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TISSUE EXTRACTS AND BLOOD COAGULATION

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The problem of whether the clotting of blood can proceed without the formation of thrombin has been the subject of considerable study for many years.

Wooldridge (1, 2) concluded that clotting is due to a reaction between two protein-lecithin compounds, one found chiefly in tissue extracts, the other in circulating blood. Mills (3, 4, 5, 6) has revived and modified this theory. He states that the normal clotting in wounds is due chiefly to a reaction between "tissue fibrinogen" (tissue coagulant), calcium and blood fibrinogen, thus excluding thrombin from the clotting mechanism. However, he recognizes that a thrombin clotting mechanism may function in the case of wounds but its action here is much slower and no doubt of little value in stopping hemorrhage.

Morawitz (7), Fuld and Spiro (8), Smith, Warner and Brinkhouse (9) found that no clotting occurred on the addition of tissue extract to purified blood fibrinogen in the presence of calcium.

In an attempt to throw additional light on this question the author approached the problem from two different angles. First, will clotting occur if any part of the thrombin mechanism is blocked out or prevented from performing its normal function; second, will *pure* tissue extract (tissue fibrinogen) plus calcium and *pure* blood fibrinogen cause fibrin formation, thus excluding the possible intervention of prothrombin or thrombin. Both prothrombin and thrombin are normally associated with blood fibrinogen and plasma and have to be removed by careful purification.

EXPERIMENTS ON CLOTTING MECHANISM. The object of this series of experiments was to determine whether clotting would occur on the addition of tissue extracts when some essential substance necessary to thrombin clotting is prevented from acting. For example, if prothrombin or

thrombin were removed from action, would the suggested tissue fibrinogen-calcium-blood fibrinogen clotting mechanism function?

The anticoagulants selected for this work were heparin, hirudin, aluminum hydroxide and magnesium hydroxide. These agents prevent blood coagulation by acting on either thrombin or prothrombin thus preventing the formation of thrombin-fibrin (clot).

Preparation of purified tissue extracts. First method: Lung tissue was freed of prothrombin by preliminary pulmonary bleeding and injections of physiological saline until the animal died. The need of perfusion to remove prothrombin was indicated by the work of Loucks and Scott (10), and Smith et al. (9). The lungs were then removed, washed, ground, and mixed well with $Mg(OH)_2$. After standing one hour the mixture was washed free of $Mg(OH)_2$ using distilled water. This procedure was then repeated with $Al(OH)_3$ to insure complete removal of prothrombin. The lung tissue was next mixed with 1 per cent NaCl and, after standing in an ice box 24 hours, filtered. An equal volume of saturated $(NH_4)_2SO_4$ was then added to the filtrate to precipitate the globulin fraction which contains the tissue coagulant. This was removed by filtration, purified by dissolution in 1 per cent NaCl, and reprecipitation by $(NH_4)_2SO_4$ five times. The precipitate of pure tissue fibrinogen containing $(NH_4)_2SO_4$ was stored in the ice box. As the product was needed the desired amounts were taken and dialyzed against saline to remove the $(NH_4)_2SO_4$. The presence of $(NH_4)_2SO_4$ tends to accelerate clotting time. The entire process except the grinding of the tissue was carried out under refrigeration (8°C.) since the extract deteriorates at ordinary temperatures.

Second method: Fresh lung tissue was treated to remove prothrombin and dried under reduced pressure at 45°C. Suitable portions were removed as needed and extracted with 1 per cent NaCl.

Preparation of thrombin. First method: The procedure described by Howell (11) was followed.

Second method: Freshly clotted blood was allowed to stand until the serum separated. The serum which separated proved a rich source of thrombin.

Preparation of thromboplastin. This was prepared from rabbits' brains and carefully purified. Thromboplastin prepared by the Cutter Laboratory was also used.

Heparin (antiprothrombin) was originally prepared by Howell and Holt (12) from dog's liver. The heparin used in these experiments was obtained from Hynson, Westcott, and Dunning, Inc. One milligram of this product prevents the clotting of 5 cc. of cat's blood for 24 hours.

Method: Five milligrams of heparin were added to 10 cc. of freshly oxalated plasma and kept at 37½°C. for 4 hours. The blood was then

centrifuged and aliquots taken. A control sample without heparin was similarly treated.

Procedure: One-half cubic centimeter aliquots of the heparin-treated oxalated plasma were placed in small glass test tubes (75 x 10 mm.) and immersed in a water bath at 38°C. To each tube were added the substances listed in tables 1 and 2 in the order given. When the last reagent, calcium chloride, was added the mouth of the test tube was covered by the thumb, the tube inverted twice and the time recorded immediately. The end point for the clotting was taken as the time when the test tube could be inverted without the blood escaping. The progress of the clotting was determined by tilting the test tube every 10 to 15 seconds toward the end of the clotting period.

The results in tables 1 and 2 show the effect of tissue extracts (T. E. 1 and 2), thrombin, cephalin, and thromboplastin on heparin-treated oxalated beef blood and heparin-treated fresh human blood, respectively. Adequate controls were also added. All clotting experiments were determined at 38°C.

The results in table 1 show that oxalated blood plasma treated with heparin and recalcified failed to clot over a period of 24 hours (no. 6). The same blood was treated with samples of tissue extract (T. E. 1 and 2), and recalcified. They also failed to clot over the same period. Thrombin or cephalin when added to similarly treated plasma resulted in clotting. Thromboplastin, on the other hand, had no effect on the clotting of heparin-treated plasma.

The following control experiments were performed: The normal clotting time of fresh oxalated plasma, the effect of tissue extracts, and the effect of thrombin on fresh oxalated plasma. The controls showed that oxalated plasma clotted normally in three minutes and fifty seconds; the tissue extracts decreased clotting time, clotting occurring in 12 and 20 seconds respectively, in numbers 8 and 9; thrombin clotted fresh blood in 10 seconds.

Table 2 records the results of adding tissue extracts, etc., to heparin-treated fresh human blood. The blood after being drawn was immediately added to heparin (5 mgm. of heparin to 10 cc. of blood) and left at room temperature for 4 hours. The blood was then centrifuged for 10 minutes and 1 mgm. more of heparin added to each 10 cc. of the clear plasma. Samples of 0.5 cc. of the treated plasma were then used.

Thromboplastin again had no coagulating effect on heparinized blood. The other results were much the same as obtained with oxalated beef blood. In both the oxalated and the fresh blood, treated with heparin, the clotting action of tissue extract was prevented.

We may assume from the above results that heparin prevents the prothrombin from forming thrombin and subsequently thrombin-fibrin or

the clot. Since no clot was formed evidently no clotting mechanism exists such as a tissue fibrinogen-calcium-blood fibrinogen mechanism.

TABLE 1

The effect of tissue extracts, thrombin, cephalin and thromboplastin on heparin-treated oxalated beef blood plasma

NUMBER	PLASMA	TREATMENT	SALINE (1 PER CENT)	ADDED	$CaCl_2$ (1 PER CENT)	CLOTTING TIME
	cc.		cc.	cc.	cc.	
1	0.50	Heparin	0.15	0.10 T. E. 1	0.05	Negative 24 hours
2	0.50	Heparin	0.15	0.10 T. E. 2	0.05	Negative 24 hours
3	0.50	Heparin	0.15	0.10 Thr.	0.05	3 minutes 30 seconds
4	0.50	Heparin	0.15	0.10 Ceph.	0.05	1 minute 57 seconds
5	0.50	Heparin	0.15	0.10 Thr. pl.	0.05	Negative 24 hours
				Controls		
6	0.50	Heparin	0.15	0.10 Sal.	0.05	Negative 24 hours
7	0.50	No heparin	0.15	0.10 Sal.	0.05	3 minutes 50 seconds
8	0.50	No heparin	0.15	0.10 T. E. 1	0.05	12 seconds
9	0.50	No heparin	0.15	0.10 T. E. 2	0.05	20 seconds
10	0.50	No heparin	0.15	0.10 Thr.	0.05	10 seconds

T. E. = purified tissue extracts. Thr. = thrombin. Thr. pl. = thromboplastin. Ceph. = cephalin.

TABLE 2

The effect of tissue extracts, thrombin, cephalin and thromboplastin on heparin-treated fresh human blood

NUMBER	PLASMA	TREATMENT	SALINE (1 PER CENT)	ADDED	CLOTTING TIME
	cc.		cc.	cc.	
1	0.50	Heparin	0.20	0.10 T. E. 1	Negative 24 hours
2	0.50	Heparin	0.20	0.10 T. E. 2	Negative 24 hours
3	0.50	Heparin	0.20	0.10 Thr.	2 minutes 20 seconds
4	0.50	Heparin	0.20	0.10 Ceph.	1 minute 20 seconds
5	0.50	Heparin	0.20	0.10 Thr. pl.	Negative 24 hours
				Controls	
6	0.50	Heparin	0.20	0.10 Sal.	Negative 24 hours
7	0.50	No heparin	0.20	0.10 Sal.	3 minutes 50 seconds
8	0.50	No heparin	0.20	0.10 T. E. 1	12 seconds
9	0.50	No heparin	0.20	0.10 T. E. 2	18 seconds
10	0.50	No heparin	0.20	0.10 Thr.	7 seconds

The *hirudin* used for these studies was obtained from Eimer and Amend. Hirudin is though to be a proteose. It inhibits fully formed thrombin in vitro or in vivo and may therefore be considered an anti-thrombin.

Method: Ten milligrams of fresh hirudin were added to 20 cc. of freshly oxalated blood plasma and left at room temperature for 4 hours. Five cubic centimeter samples were then taken for the experiment.

Procedure: The procedure used was similar to that employed in the experiment with heparin.

The results presented in table 3 show the effects of tissue extracts, thrombin, cephalin, and thromboplastin on the clotting time of hirudin-treated oxalated blood plasma. The addition of tissue extracts or thromboplastin to hirudin-treated oxalated plasma failed to result in clot formation after recalcification. However, when thrombin was added to a similar sample of plasma clotting occurred in 8 minutes, 30 seconds. The addition of cephalin likewise resulted in clotting in 4 minutes, 30 seconds.

TABLE 3

The effect of tissue extracts, thrombin, cephalin and thromboplastin on the clotting time of hirudin-treated oxalated blood plasma

NUMBER	PLASMA	TREATMENT	SALINE (1 PER CENT)	ADDED	$CaCl_2$ (1 PER CENT)	CLOTTING TIME
	cc.		cc.	cc.	cc.	
1	0.50	Hirudin	0.15	0.10 T. E. 1	0.05	Negative 24 hours
2	0.50	Hirudin	0.15	0.10 T. E. 2	0.05	Negative 24 hours
3	0.50	Hirudin	0.15	0.10 Thr.	0.05	8 minutes 30 seconds
4	0.50	Hirudin	0.15	0.10 Ceph.	0.05	4 minutes 30 seconds
5	0.50	Hirudin	0.15	0.10 Thr. pl.	0.05	Negative 24 hours
				Controls		
6	0.50	Hirudin	0.15	0.10 Sal.	0.05	Negative 24 hours
7	0.50	No hirudin	0.15	0.10 Sal.	0.05	3 minutes 20 seconds
8	0.50	No hirudin	0.15	0.10 T. E. 1	0.05	14 seconds
9	0.50	No hirudin	0.15	0.10 T. E. 2	0.05	12 seconds

The results show that hirudin prevented the clotting action of thrombin. Since clotting did not occur even though the elements identified with the suggested second clotting mechanism were present we have additional proof that no such mechanism exists.

Aluminum cream ($Al(OH)_3$) was prepared according to the directions given in Hawk and Bergheim (13). Bancroft et al. (14) have shown that its anti-coagulating action is due to the removal of prothrombin.

Method: One-half cubic centimeter of $Al(OH)_3$ was added to fresh oxalated blood plasma, thoroughly mixed, and allowed to stand for 3 hours at $37\frac{1}{2}$°C. After centrifuging for 10 minutes aliquots of the decanted plasma were used in the following experiments, table 4.

The effects of the addition of tissue extracts, thrombin, and thromboplastin on the clotting time of oxalated plasma after treatment with

$Al(OH)_3$ and recalcification are given in table 4. Tissue extracts and thromboplastin resulted in failure to clot in 24 hours. Thrombin, on the other hand, caused clotting in 2 minutes and 40 seconds. Suitable controls showed that the untreated oxalated plasma clotted in 24 seconds and

TABLE 4

The effect of tissue extracts, thrombin and thromboplastin on the clotting time of $Al(OH)_3$ treated oxalated plasma

NUMBER	PLASMA	TREATMENT	SALINE (1 PER CENT)	ADDED	$CaCl_2$ (1 PER CENT)	CLOTTING TIME
	cc.		cc.	cc.	cc.	
1	0.50	$Al(OH)_3$	0.15	0.10 T. E. 1	0.05	Negative 24 hours
2	0.50	$Al(OH)_3$	0.15	0.10 T. E. 2	0.05	Negative 24 hours
3	0.50	$Al(OH)_3$	0.15	0.10 Thr.	0.05	2 minutes 40 seconds
4	0.50	$Al(OH)_3$	0.15	0.10 Thr. pl.	0.05	Negative 24 hours
			Controls			
5	0.50	$Al(OH)_3$	0.15	0.10 Sal.	0.05	Negative 24 hours
6	0.50	No $Al(OH)_3$	0.15	0.10 Sal.	0.05	3 minutes 50 seconds
7	0.50	No $Al(OH)_3$	0.15	0.10 T. E. 1	0.05	24 seconds
8	0.50	No $Al(OH)_3$	0.15	0.10 T. E. 2	0.05	30 seconds

TABLE 5

The effects of tissue extracts, thrombin and thromboplastin on the clotting time of $Mg(OH)_2$ treated oxalated plasma

NUMBER	PLASMA	TREATMENT	SALINE (1 PER CENT)	ADDED	$CaCl_2$ (1 PER CENT)	CLOTTING TIME
	cc.		cc.	cc.	cc.	
1	0.50	$Mg(OH)_2$	0.15	0.10 T. E. 1	0.05	Negative 24 hours
2	0.50	$Mg(OH)_2$	0.15	0.10 T. E. 2	0.05	Negative 24 hours
3	0.5	$Mg(OH)_2$	0.15	0.10 Thr.	0.05	2 minutes 20 seconds
4	0.5	$Mg(OH)_2$	0.15	0.10 Thr. pl.	0.05	Negative 24 hours
			Controls			
5	0.5	$Mg(OH)_2$	0.15	0.10 Sal.	0.05	Negative 24 hours
6	0.5	No $Mg(OH)_2$	0.15	0.10 Sal.	0.05	10 minutes 10 seconds
7	0.5	No $Mg(OH)_2$	0.15	0.10 T. E. 1	0.05	14 seconds
8	0.5	No $Mg(OH)_2$	0.15	0.10 T. E. 2	0.05	24 seconds

30 seconds respectively, with the two different tissue extracts. Evidently the removal of prothrombin by the $Al(OH)_3$ resulted in the failure to clot formation. Since the elements for the so called tissue fibrinogen-calcium-blood fibrinogen clotting mechanism were present and no clotting occurred one was again led to believe that no such clotting mechanism exists.

Magnesium hydroxide ($Mg(OH)_2$) was chosen for its anti-prothrombin action and prepared according to the method described in U. S. P. XI (15).

Method: To 5 cc. of fresh oxalated blood plasma was added 0.5 cc. of milk of magnesia, the mixture shaken and left at 37½°C. for three hours. After centrifuging for 15 minutes the resulting plasma was used in the experiments recorded in table 5.

The results obtained with $Mg(OH)_2$ are essentially the same as those obtained with $Al(OH)_3$, i.e., on the removal of prothrombin by $Mg(OH)_2$ no clotting occurred.

Occasionally during the experiments using $Al(OH)_3$ and $Mg(OH)_2$ treated oxalated plasma a small thread of clot would form. This was no doubt due to incomplete removal of all the prothrombin by the treatment employed.

Experiments using purified clotting elements. The object of this experiment was to observe whether clotting would occur when purified tissue extracts, purified fibrinogen, and calcium were brought together.

TABLE 6

Action of "tissue fibrinogen" on blood fibrinogen

NUMBER	BLOOD FIBRINOGEN	$CaCl_2$	SUBSTANCES ADDED	CLOTTING TIME
	cc.	cc.		
1	0.50	0.05	Saline	Negative 24 hours
2	0.50	0.05	Thrombin	35 seconds
3	0.50	0.05	T. E. 1	Negative 24 hours
4	0.50	0.05	T. E. 2	Negative 24 hours
5	0.50	0.05	T. E. (crude)	3 minutes 00 seconds

The method for the preparation of pure tissue extracts has been given. Purified blood fibrinogen was prepared as follows: 30 cc. of fresh oxalated blood plasma were treated with 7 cc. of $Mg(OH)_2$, left for 3 hours then centrifuged for 10 minutes. To the clear plasma was added one-third of its volume of saturated $(NH_4)_2SO_4$, the substances mixed well and allowed to stand for a few minutes. The precipitate was purified by repeated dissolution in 1 per cent saline followed by reprecipitation with an equal volume of saturated $(NH_4)_2SO_4$. Six such purifications were carried out. The final $(NH_4)_2SO_4$ precipitate of tissue extract was dialyzed against 0.9 per cent NaCl for 6 hours. No adjustment of pH could be made in view of the danger of precipitating the tissue extract.

The results found in table 6 show that when pure tissue extract, "tissue fibrinogen," was added to pure blood fibrinogen in the presence of calcium no clotting occurred. However, when thrombin was added to blood fibrinogen clotting occurred immediately. A crude lung extract was

prepared by extraction with 1 per cent NaCl, followed by precipitation with $(NH_4)_2SO_4$ and subsequent removal of the $(NH_4)_2SO_4$ by dialysis. This likewise caused the clotting of purified blood fibrinogen.

These experiments show that the unpurified constituents in contrast to the purified products result in clot formation. Evidently in the case of the former the impurities such as thrombin or prothrombin are responsible for the clotting action. This conclusion is further substantiated by the fact that the addition of thrombin to the purified products results in clot formation.

CONCLUSIONS

Blood clotting does not occur when a part of the thrombin mechanism is interfered with in spite of the fact that the necessary constituents for the proposed tissue fibrinogen-calcium-blood fibrinogen mechanism are present. We may therefore conclude that the latter mechanism is not responsible for clot formation.

No clot formation occurred when purified tissue extract-calcium and purified blood fibrinogen were used. On the other hand, when unpurified products were used clot formation resulted. These findings lead one to believe that impurities in the reagents may have been responsible for the proposal of a tissue fibrinogen-calcium-blood fibrinogen clotting mechanism.

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EFFECTS OF FARADIC STIMULATION OF THE CEREBRAL CORTEX ON LIMB AND RENAL VOLUMES IN THE CAT AND MONKEY[1]

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Dusser de Barenne and Kleinknecht (1) observed falls of blood pressure following electrical stimulation of the motor areas of dogs, cats, and rabbits anesthetized with ether, urethane or morphine. Later Hoff and Green (2) found that rises of blood pressure could be obtained from similar cortical areas of cats and monkeys when anesthetized with ether. Since mean blood pressure as recorded in these experiments represents only the resultant of simultaneous vascular changes in different parts of the body, the problem of whether a rise or a fall in pressure occurs may depend upon various factors such as anesthesia and the character of cerebral representation of the vasomotor system. The problem has therefore been investigated afresh by combining studies of blood pressure with measurement of organ volumes.

METHODS. Bilateral limb volumes, renal volume, and blood pressure were optically recorded while stimulating the cortex of 14 cats and 14 monkeys anesthetized with ether, or with Dial-Ciba (containing urethane) supplemented with ether. While curare was usually given in addition to paralyze muscular movements, a few experiments were performed without this drug to demonstrate that volume changes were obtainable in the absence of normal muscular movement with stimuli below the threshold for muscular response. In all cases in which curare was administered, artificial respiration was maintained throughout the experiment.

The limb plethysmographs were closed at the upper end by rubber diaphragms made of dental dam pierced with a hole large enough to admit the limb, without obstructing the venous return, and were adjusted so as to include the fore-arms and as much of the upper arms as possible. An air-tight seal was obtained by shaving or closely clipping the hair and cementing the rubber dam to the arm with gum-rubber cement. The plethysmographs were filled with water at 35–40°C., except in those instances in which the limbs were skinned when Ringer-Locke's solution was used. The changes in volume were recorded with Frank segment capsules (3) covered with condom rubber, using air transmission. The use of a combined air-fluid transmission system introduces certain artefacts, namely, extraneous fine vibrations which may be noticed in some records, but these do not interfere with the

[1] A preliminary report of these experiments was communicated to the American Physiological Society, March 26, 1936.

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interpretation since gross changes only, and not the contour, are being studied. This air-fluid system was used because it greatly diminished shifting of the base level of the volume curves due to changing temperature of the air contained within the plethysmographs. Renal volume was recorded with an oncometer composed of two shallow cups hinged together covered with light rubber dam and arranged to surround the kidney without constricting the pedicle. The oncometer halves were filled with either water or air and connected by air-filled tubes to a Frank segment capsule.

In nearly all instances, blood pressure was recorded from a femoral artery with a Wiggers optical manometer (4), using paraffin-coated glass cannulae and boiled 20 per cent sodium sulphate as the anticoagulant. The manometer and cannula had a natural frequency of 75 to 100 per second. A constant slow intravenous infusion of mammalian Ringer-Locke's solution was maintained throughout all experiments. Although in a few instances a thyratron stimulator was used, the stimulating current was usually obtained from a 1100 ohm potentiometer shunted across the standard 60 cycle 110 volt alternating current. The potential across the stimulating electrodes could be readily adjusted within 0.1 volt over the range of 0 to 110 volts. Bipolar electrodes separated about 1.0 mm. were used, the cortex being covered with warm, moist pledgets between stimuli which were applied not oftener than once in 2 minutes and lasted 5 to 15 seconds.

RESULTS. *1. Either a rise or a fall of mean blood pressure may result from the stimulation of the motor area of the cortex.* In a previous paper (2) it was demonstrated that under ether anesthesia, plus curare to prevent muscular activity, only a rise of blood pressure could consistently be obtained from the motor area of the cat's cortex and from the motor and premotor areas of that of the monkey. This observation has been confirmed in the course of these experiments but, in addition, it has been observed that in animals anesthetized by Dial supplemented by ether, no change, or a fall of mean blood pressure more often resulted from excitation of the same areas of the cortex (see fig. 2, A–D). This latter observation therefore confirms Dusser de Barenne and Kleinknecht's findings that decline of pressure may be elicited by stimulation of the motor cortex of animals.

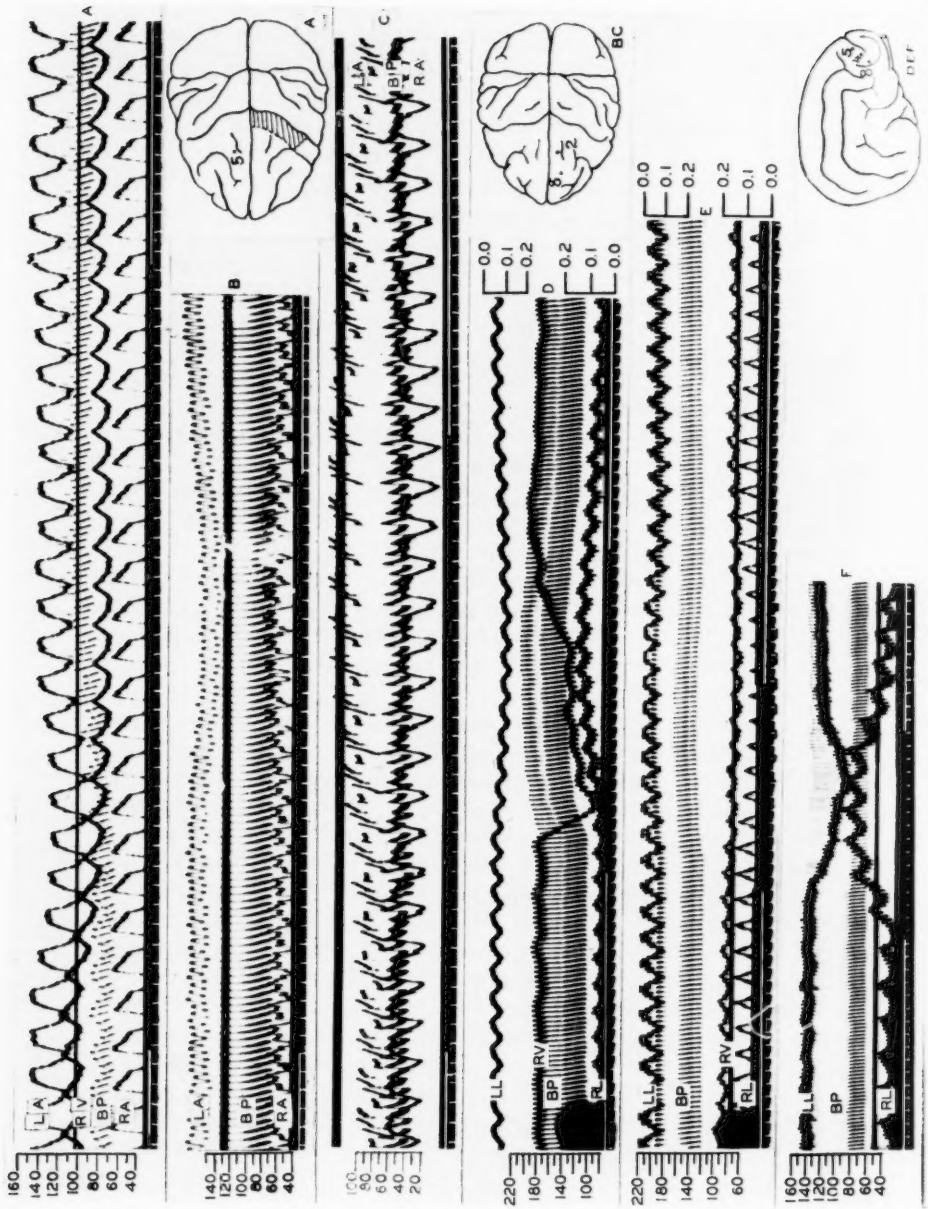
2. The blood flow in different regions of the body as measured by alteration of the volume of these regions, is affected by stimulation of the cortex. a. *Renal volume is usually decreased.* A diminution of renal volume consistently occurs in curarized animals under ether anesthesia during the rise of blood pressure evoked by stimulation of the motor cortex. A typical record of such a response is reproduced in figure 1D. In this and all subsequent records, the kidney volume is labeled RV and downward movement of the line indicates a decrease in volume. The diminution of renal volume slightly precedes the rise of blood pressure. In a series of experiments this amounted to 0.5 to 1.0 second, the latency of the renal volume changes varying from 1.5 to 3.5 seconds and that of the simultaneously recorded blood pressure from 2.0 to 4.5 seconds. An equivalent change in volume of a given kidney occurred following stimulation

of either cerebral hemisphere. After sectioning the nerves to the kidney on one side, that kidney now dilated during the rise of mean blood pressure. That the rise of blood pressure is not dependent solely on vascular constriction in the viscera was shown in two experiments by a considerable rise of pressure even after complete abdominal evisceration.

b. *Limb volume is usually increased.* Stimulation of the sigmoid gyrus of the cat's cortex, and of areas 4 and 6 in the monkey produced in almost every instance an alteration of limb volume. The most frequent response in both cat and monkey was a dilatation of the limbs occurring after a latency of 1.5 to 13 seconds with an average latency of 4.1 seconds. Very weak stimuli (as low as 0.8 volt A.C. 60 cycle) were often more effective than stronger stimuli. This dilatation was greatest about 9 seconds after onset of stimulation and occurred regardless of any alteration of the mean blood pressure. Thus in figure 1D, a record obtained under ether anesthesia, the limbs dilated during a rise in blood pressure while, as illustrated in figure 1B, dilatation occurred in the absence of change of blood pressure and in figure 2, A–D, during a decline of blood pressure. In the experiments from which the last two figures were taken, Dial anesthesia was used. In these as in all subsequent records, the limb volume curves are labeled LL or LA for the left extremity and RL or RA for the right. Approximation of the limb volume curves, i.e., downward movement of the line for the left extremity and upward movement of that for the right, indicates increased volume of the extremities. Usually three waves may be seen in each record: a small one, the pulse wave; a slightly larger and slower one, the respiratory wave, and the large slow wave indicating the change of volume or of pressure as a result of the stimulation.

c. *Limb volume is occasionally diminished.* While stimulation of the motor areas both of monkeys and cats anesthetized with ether usually produced a dilatation of the extremities, in 5 monkeys and 1 cat, anesthetized with Dial, stimulation of similar areas frequently resulted in a constriction of the limbs with a latency of 1.5 to 11 seconds (average 6.0 sec.). This constriction was usually accompanied by a rise of blood pressure, although occasionally there was no change or even a slight fall of blood pressure. Records showing constriction of limbs during stimulation of the cortex are reproduced in figures 1E; 3A–B, and 4A.

Either constriction or dilatation of the limbs was followed by return to normal approximately 8 to 13 seconds after the end of stimulation. Usually no after-response was encountered although in some instances excitation of the cortex produced an initial constriction of the limbs followed by a delayed dilatation which became more marked just after cessation of the current. In all experiments the alteration of limb volumes during stimulation occurred in the same direction on both sides of the body. The latencies of the response were equal as were the durations of the


A
LA
RV
BP
RA
B
C
BC
D
E
F
LL
RL
0.0
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return to the prestimulation level. In many experiments, a comparison was made of the changes in volume of a pair of extremities in response to alternate stimulation of corresponding points on the right and left motor areas. The upper two records of figure 2 illustrate the method of studying this phenomenon. Record A of excitation of point 5 on the right cortex (see map of cortex accompanying record) demonstrates slightly greater dilatation of the right leg, and B of stimulation of the corresponding point on the left cortex, greater dilatation of the left leg, the two records indicating greater alteration on the ipsilateral side. In the cat this was no more frequent than a response stronger on the contralateral side, but in 5 monkeys the most frequent response was greater dilatation in the contralateral extremities.

3. The direction and extent of the alteration of the blood pressure is dependent on the resultant of the various changes in flow in the different parts

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Fig. 1. Record A. Dilatation of the limbs with reduction of kidney volume without change of blood pressure succeeded by diminution of the volume of the limbs and a rise of blood pressure. The point stimulated is indicated on cortical map A by a numbered dot adjacent to the anterior end of the superior precentral sulcus. *Macaca mulatta*, female, weight 4.7 kgm., ether anesthesia, curare 18 mgm., A. C. 60 cyles 15 volts, artificial respiration. Top record *LA*—left arm, ↓ = increased volume; second record *RV*—renal volume ↑ = increased volume; third record *BP*—blood pressure, calibration at left side of record; bottom record *RA*—right arm ↑ = increased volume; horizontal white line—stimulation signal. Interruption of this line indicates period of stimulation; small vertical white lines—time, 1.0 second. Expt. C. S. 42–40a–5R.

Records B and C. Effect of initial blood pressure on limb volume response to cortical stimulation in a macaque. Record B, dilatation of the arms with a pressure of 130/70 mm. Hg from stimulation of point 8; location of points indicated on cortical map *B*, *C*. Female, weight 4.5 kgm., anesthesia—Dial-Ciba (with urethane) 2.7 cc. intraperitoneally supplemented by ether, artificial respiration, saline infusion, Harvard inductorium—coil 11 cm. and 8 cm. respectively. Lettering same as Record A. Expt. C. S. 58–8–2L; 43–8L.

Record D. Simultaneous dilatation of the forelimbs, reduction of the renal volume and elevation of blood pressure from stimulation of the extreme lateral part of the posterior sigmoid gyrus of the cat. Point 8 on cortical map *D*, *E*, *F*. Male, weight 2.7 kgm., anesthesia—ether, curare 39 mgm., artificial respiration, saline infusion, A. C. 60 cycle 3.5 volts. *LL*—left foreleg, ↓ = increased volume; *RL*—right foreleg ↑ = increased volume, calibrations on right—magnitude of volume change in cubic centimeter; other lettering as in record A. Expt. C. S. 65–13–8R.

Record E. Reduction of renal and limb volumes with rise of pressure from stimulation of the posterior sigmoid gyrus of the same cat from which record D was taken. Point 4 on cortical map *D*, *E*, *F*. A. C. 60 cycle 3 volts. Lettering same as record D. Expt. C. S. 65–6–4R.

Record F. Dilatation of limbs without movement from stimulation of the posterior sigmoid gyrus of a non-curarized cat anesthetized with ether. Point 5 on cortical map, *D*, *E*, *F*. Male, weight 3.5 kgm., A. C. 60-cycle 1.75 volts, intravenous infusion Ringer-Locke's solution, lettering same as record D. Expt. C. S. 64–6–5R.

of the body. In those conditions in which cortical stimulation produced no change in systemic blood pressure, the limb and kidney volumes varied reciprocally and the duration of the changes was approximately the same in each organ. This same relationship was also often present when the pressure fell or when it rose. Figure 1D is an example of the latter condition. In many instances both the limbs and kidney were constricted, and in these the blood pressure always rose. Figure 1E is an example of these phenomena. Dilatation of both the kidney and the limbs was never observed. In one experiment a fortuitous occurrence (recorded in fig. 1A) demonstrates clearly that the mean blood pressure is the resultant of somatic and visceral vascular reactions. The initial effect of cortical stimulation was dilatation of the extremities with constriction of the kidneys, the net effect being no change in blood pressure. Approximately 11 seconds after the onset of stimulation the limbs began to diminish in volume while the kidney remained constricted and the blood pressure began to rise.

4. Evidence that the vascular changes are active and not passive phenomena. Weber (5), observing the effect of cortical stimulation on blood pressure, limb, and gut volume recorded simultaneously, found that the rise in blood pressure was followed a few beats later by a slight increase in the volume of both limbs while a great reduction of gut volume occurred with the elevation of pressure. He concluded at that time that this rise in blood pressure resulting from cortical stimulation was due mainly to constriction of vessels in those viscera innervated by the splanchnic nerves and that the increase in limb volume was a passive enlargement associated with increased systemic blood pressure. Later, however, Weber (6) found that the increase in limb volume after cortical stimulation was greater following evisceration than before, despite the fact that the rise in pressure was less after this procedure. He was therefore led to revise his previous opinion and to suggest an active dilatation in the vessels of the limbs. That the somatic vascular areas do participate actively in the change of systemic blood pressure and the redistribution of blood in the body induced by cortical stimulation and that the volume changes are mediated through nervous channels is substantiated in our own experiments by the following lines of evidence:

a. *Variations of limb volume are independent of blood pressure changes.* In the earlier experiments, using ether anesthesia, increase in limb volume was consistently obtained in both cats and monkeys, but was always associated with a rise of pressure. However, under Dial anesthesia the blood pressure either rose negligibly or not at all, or fell during cortical stimulation, while the limb volume showed as great a dilatation as with ether. Although it is quite possible that the dilatation of limbs with increase of pressure recorded in the ether experiments may have been

passively produced, the degree of dilatation was usually greater than that occurring with a simple rise of pressure; furthermore it is difficult to conceive of an increase of limb volume during a declining pressure except as a result of an active relaxation of the vascular bed of the limbs. Moreover, under certain conditions the limbs have definitely constricted during a rise of pressure, and again in these circumstances such an occurrence is impossible except as a result of active constriction of the vessels of the limbs. Finally the diminution of the volume of the extremities is not a result of shunting of blood to other parts of the body such as the abdominal viscera, since renal volume records usually indicated a simultaneous constriction of the renal vascular bed.

b. *Limb volume changes persist after adrenalectomy.* In one cat and one monkey in which the adrenal arteries and veins had been ligated and the adrenal glands removed, active changes of limb volume continued to occur, so that it does not seem likely that they are due to the liberation of adrenalin as a result of stimulation of the cerebral cortex. Furthermore, as shown in figure 4C, injection of adrenalin produced a marked dilatation of an extremity of a monkey in which cortical stimulation had just previously yielded a reduction of limb volume.

c. *Cortically induced changes in limb volume are abolished by denervating an extremity.* In one monkey showing constriction of the forelimbs with rise of pressure on cortical stimulation, the right extremity was denervated by section of the nerves in the axilla. After the section, stimulation of the cortex produced a slight passive dilatation in the denervated arm with the rise of pressure, while the volume of the normal arm was diminished. The denervated arm responded to intravenous injection of adrenalin as did the normal by dilatation associated with a greatly delayed rise of pressure but an immediate fall of kidney volume (see fig. 4A–C). A similar procedure was carried out in another monkey in which cortical stimulation produced a dilatation of the forelimbs with fall of blood pressure. Upon denervation of the left forelimb by section of the nerve trunks in the axilla, this extremity decreased in volume as the blood pressure fell while the right extremity continued to dilate during stimulation of the cortex. These experiments indicate that the variations in peripheral vascular resistance produced by cortical stimulation are not mediated by any humoral agent liberated in other parts of the body and are not passive effects due to alteration in general blood pressure, but are caused by nerve impulses passing to the vascular area from which the responses are obtained.

5. The muscles of the extremities participate in the limb volume dilatation. In order to examine directly the changes in volume which occur in the muscle as a result of cortical stimulation, limb volumes have been recorded before and after skinning and compared with records obtained simul-

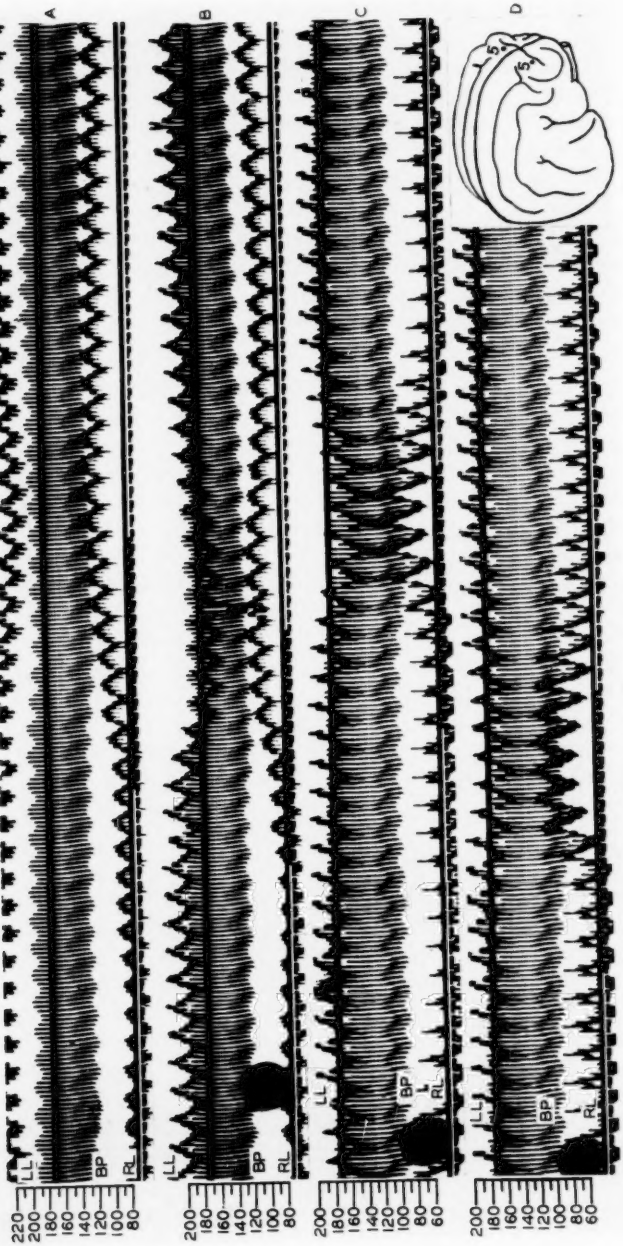

Fig. 2

taneously from the opposite normal extremity. In one cat in which stimulation of the posterior sigmoid gyrus yielded a decline in pressure and simultaneous dilatation of the forelimbs, the response after complete removal of all the skin from that part of a forelimb included in the plethysmograph still took place with the same latency and the same duration in comparison with the opposite normal extremity. This is illustrated in the records given in figure 2A–D. Stimulation of the motor area on either side in 2 cats still produced bilateral forelimb dilatation after removing the skin from both limbs (fig. 3C and D). Similarly vasoconstriction associated with no change or a rise in pressure was obtained in cats both before and after skinning the limbs (fig. 3A and B). Inasmuch as these alterations in volume recorded from skinned limbs are active changes, and since in every case the blood pressure varied in the opposite direction to the volume change, it is concluded that the variations in limb volume are at least partly if not chiefly attributable to an active vasodilatation or vasoconstriction in the muscles.

6. Limb volume responses occur without epileptic muscular contractions. It was previously shown (2) that alterations of blood pressure resulting from cortical stimulation can occur in non-curarized animals in the absence of simple muscular response or epileptic seizures. A similar conclusion may be reached with regard to limb volume changes. They have been produced without the slightest accompanying muscular movements in curarized animals. They occur with short latency and the limb volumes return to the prestimulation level shortly after the end of stimulation with no suggestion of an after-response. Furthermore in lightly etherized non-curarized animals, well-marked volume changes were evoked with cortical stimuli so weak as to be below the threshold for somatic muscular responses (see fig. 1F). In the same animals, slightly more intense stimuli evoked discrete muscular movements of the opposite side.

7. Factors affecting the vascular response. In a given animal under constant experimental conditions successive stimulation of various points in the motor and premotor cortex of monkeys and of the sigmoid gyri

Fig. 2. Limb volume changes elicited from stimulation of corresponding areas on the right and left posterior sigmoid gyri of the cat before and after removing the skin from one of the extremities. Fall of pressure from stimulation of these areas under dial anesthesia. Records A and B, stimulation of right and left cortex respectively before skinning; and records C and D, the same areas after skinning the left forelimb. Points of stimulation indicated by numbered dots on cortical map. Male, weight 3.0 kgm., anesthesia—Dial-Ciba (with urethane) 1.5 cc. intraperitoneally supplemented by ether, curare 30 mgm., artificial respiration, intravenous infusion Ringer-Locke's solution, A. C. 60-cycle 1.5 volts for A and B, and 1.25 volts for C and D. Lettering same as in figure 1, record D. Expt. C. S. 67-5-5R; 6-5L; 19-5R; 20-5L.

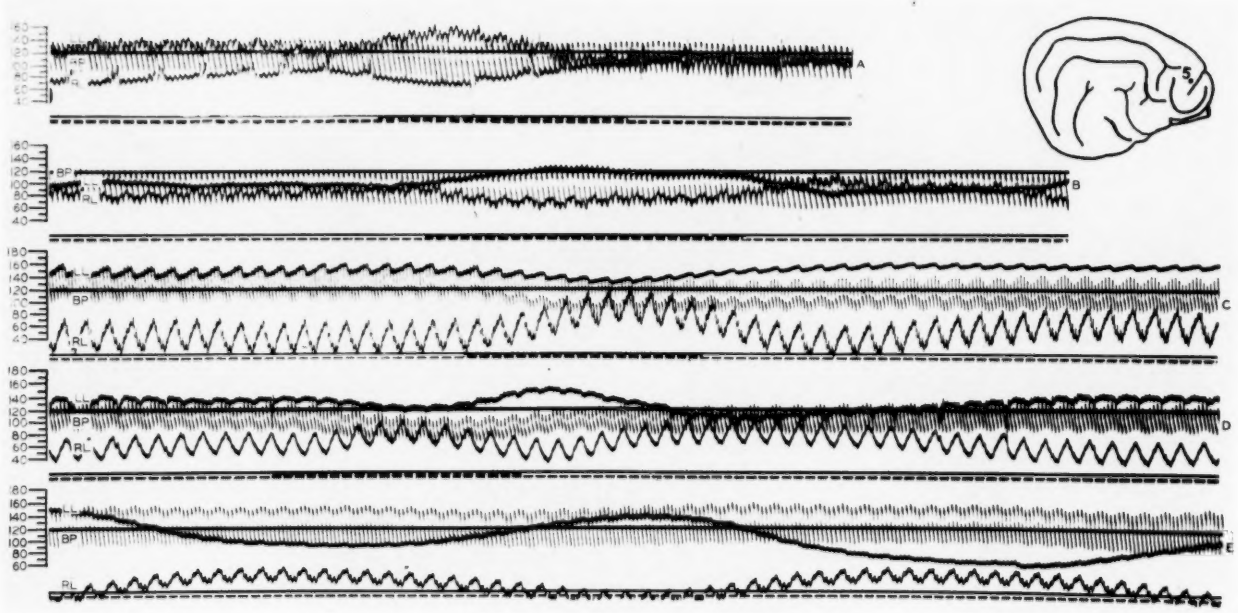

Fig. 3

and gyrus proreus of cats has yielded a similar result from each point. This result may be any of the combinations of vascular change previously referred to, namely, dilatation or constriction of the limbs with fall or rise or no change of pressure. However there are a number of factors which appear to affect the direction and extent of these changes: 1. In three instances weak stimuli, 0.8 to 2.0 volts A. C. 60-cycle, evoked a dilatation of the limbs but with stronger stimuli there was constriction of the limbs. 2. In six experiments in which cortical stimulation produced constriction of the limbs, the diminution of volume occurred most frequently when the systolic blood pressure ranged from 60 to 100 mm. Hg (see fig. 1C and fig. 4A), but rarely with a pressure above 140 (fig. 1E). Dilatation of the limbs on the other hand occurred at all ranges of pressure. 3. In 2 experiments in which cortical stimulation was consistently producing dilatation of the limbs associated with a considerable fall of pressure, diminution of the depth of the artificial respiration converted the response to a reduction of limb volume with a slight fall of pressure. After restoration of the pulmonary ventilation to its original depth, cortical stimulation again gave rise to dilatation of the limbs and a considerable fall in the blood pressure. Records from one of these experiments are reproduced in figure 3, A–D. 4. The difference in the effects of Dial and ether anesthesias on the vascular responses has already been described (fig. 2A and B; fig. 3C; fig. 1D). 5. Toward the end of prolonged experiments, stimulation of the cortex with weak stimuli (1.5 to 2.5 volts, A. C.) frequently resulted in a dilatation of the extremities which was followed by alternate constriction and dilatation of the limbs, passing through one or more cycles before becoming stabilized. Usually the extent of the blood pressure shift was considerable only during the initial

Fig. 3. Limb volume responses from stimulation of a point on the posterior sigmoid gyrus of a cat anesthetized with Dial. Records taken in the following order —C, A, B, D, E.

Record C. Dilatation of limbs with fall of pressure.

Record A. Diminution of limb volumes elicited from cortical stimulation during a period of reduced pulmonary ventilation.

Record B. Illustrates reduction of limb volumes, taken after removal of the skin from the extremities, evoked by cortical stimulation during a period of reduced pulmonary ventilation, compare with record A.

Record D. Taken after removal of the skin from the extremities and restoration of the normal volume of pulmonary ventilation. Dilatation of the limbs followed by a short interval of periodic oscillation of limb volumes occurring late in the experiment after removing the skin from the two extremities. Point of stimulation indicated by the numbered dot on the cortical map. Female, weight 2.7 kgm., anesthesia—Dial-Ciba (with urethane) 1.4 cc. intraperitoneally supplemented by ether, curare 34 mgm., artificial respiration, intravenous infusion Ringer-Locke's solution, A. C. 60-cycle 2.5 volts. Lettering same as in figure 1, record D. Expt. C. S. 70-35-5R; 37-5R; 33a-5R; 39c-5R.

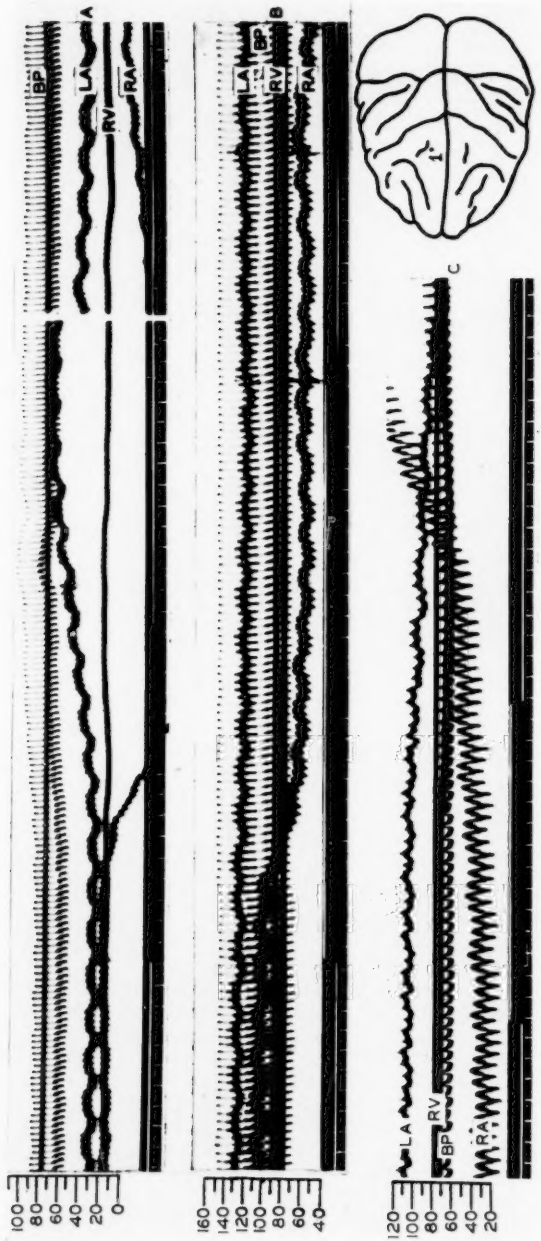
A
BP
LA
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Fig. 4

dilatation of the limbs (see fig. 3D). This tendency toward instability became very pronounced in experiments in which the skin had been removed from a pair of extremities and in one experiment stimulation of the cortex had to be discontinued shortly after removal of the skin because of the onset of a constant rhythmic dilatation and constriction of the limbs. A record showing a series of these oscillations in the volumes of skinned limbs is given in figure 3E.

DISCUSSION. In clinical literature[2] one finds numerous reports of alteration of skin temperature and the presence in hemiplegic limbs of edema. In early experimental literature Eulenburg and Landois (12, 13) observed cooling of the opposite side of the body on stimulation of the dog's cortex. Raudnitz (14) obtained the same result but found it necessary to use stimuli of an intensity which evoked an epileptic seizure. Kennard (8) has recorded, during warming and cooling of the body, differences in the skin temperature of opposite extremities of monkeys and chimpanzees which had had unilateral ablation of motor and premotor areas of the cortex.

While these observations appear to implicate the cortex in the integration of vasomotor reactions in the skin, doubt has been thrown on them by certain recent clinical studies. Thus Zollinger in 1935 (15) studied the temperature of the skin in a patient with a tumor of the left frontal lobe, and failed to find any significant differences in opposite sides of the body, even after ablation of one cerebral hemisphere (death from meningitis seventeen days after operation). Uprus, Gaylor, Williams and Carmichael (16, see also 20) simultaneously recorded the temperature of the skin of three limbs during warming and cooling of the fourth extremity. In their records on long standing hemiplegic patients, the shift of temperature was of the order of 11 to 14°C. in the finger and 0.9 to 1.1° in the rectum. Since there was no difference in the rate of warming and cooling of opposite extremities in these patients, they concluded

[2] For references see 2, 7, 8, 9, 10 and 11.

Fig. 4. Effect of denervation on limb volume responses to stimulation of a point adjacent to the superior precentral sulcus in a macaque.

Record A. Constriction of arms with rise of blood pressure.

Record B. Taken after complete denervation of the left arm. Constriction of normal right arm, slight passive dilatation of left arm with small rise of blood pressure.

Record C. Marked dilatation of both the normal right and the denervated left arms produced by intravenous injection of 2 cc. of 1:10,000 solution of epinephrine. Female, weight 3.4 kgm., anesthesia—Dial-Ciba (with urethane), 0.68 cc. intraperitoneally supplemented by ether, curare 15 mgm., A. C. 60-cycle 3 volts for A and 8 volts for B, artificial respiration, saline infusion. Lettering same as in figure 1, record A. Expt. C. S. 61-7-1R; 33-1R; 42.

that lesions of the cerebral hemipheres do not alter the functional capacity of the vessels of the hemiplegic limb to constrict or dilate in response to sensory stimuli or to changes in temperature of the blood.

During the course of the experiments presented in this paper, skin temperatures were recorded from seven monkeys with unilateral lesions of the motor or premotor cortex. In four of these, the temperature of the paralyzed extremity lagged behind the normal when warming the animal after a period of cooling which had been sufficient to lower the rectal temperature 3–4°C. and the temperature of the skin 10–15°C., an observation in harmony with those of Kennard (8).

While such data indicate that the cortex is concerned in the finer adjustment of the body to changes in environmental temperature, they do not permit analysis of the physiological rôle that the cerebral cortex plays in the control of the circulation of the body as a whole. Goldstein (17) has studied this problem by measurement of blood pressure in the extremities of patients with various cerebral lesions. In his subjects, the blood pressure in the paralyzed side was higher than in the normal extremity. Kahler (18) also found in 55 patients with cerebral tumors (16 of which came to autopsy) that the blood pressure in the affected extremities was 10 to 20 mm. higher than in the normal. These last observations suggest a difference in the blood flow in the two sides of the body in the presence of cerebral lesions.

More pertinent experimental and clinical studies have utilized registration of limb and organ volumes. The earliest studies of this type were made by Weber (5, 6). On the basis of cortical stimulation experiments in animals and suggestion of muscular movement to hypnotized subjects in whom no movement was actually made, he arrived at the conception that the cerebral cortex exerts an influence on the distribution of blood in the body. He found that the limbs dilated bilaterally as a result of cortical stimulation and therefore concluded that the observations of a difference in the temperature response on the two sides to cortical stimulation must be incorrect. Danielopolu, Radovici, Carnial and Aslan (19) studying the limb volume changes in patients with capsular hemiplegia or cortical lesions, observed that the affected extremity at some time showed conspicuous volume changes not present in the normal limb; at a subsequent examination very slight pulsations synchronous with respirations were recorded. They postulated the existence of cortical zones which normally influence the lower vegetative centers, and concluded from their experiments that release of this control by cortical lesions results in a state of exaggerated automatism which permits the limbs to pass from time to time from a condition of extreme vasodilatation to one of extreme vasoconstriction. Stürup, Bolton, Williams and Carmichael (20) in a study of finger volumes found less conspicuous

differences in responses to cold, pressure, pain, noise, deep breathing, altered blood temperature, visceral, mental activity, etc.

Since it has been shown that changes in external temperature result in a difference in the skin temperature on the two sides of the body of animals deprived of a part of one cerebral hemisphere, and since stimulation of the cerebral cortex appears to produce an alteration of skin temperature greater on one side than on the other, it might be expected that the limb volume response to cortical stimulation would also be greater on one side than on the other. However since the changes of limb volume as a result of cortical stimulation were only slightly different on the two sides, since the responses were but little diminished by skinning and since the alterations in volume remained bilateral, and the duration, extent and direction of the responses were the same in the skinned and normal extremities, it is concluded that the greater part of the volume change is due to alterations in the blood supply of the muscles and that therefore any asymmetry in the response in the skin vessels is masked by the variations in the volume of the muscles. Studies of finger volume are thus less significant than measurements of forearm volumes (cf. 20).

In our experiments, alteration of cardiac output has not been measured but evidence has been presented that a change in peripheral vascular resistance occurs in various parts of the body which may account for the modifications of blood pressure. Thus a decrease in renal volume slightly preceded the general rise in blood pressure and persisted approximately as long as the pressure rise from cortical stimulation. The increased peripheral resistance necessary for such rise of blood pressure was not limited to the abdominal viscera, for a considerable rise still occurred during cortical stimulation after removal of the adrenals and complete abdominal evisceration. In most instances of stimulation of the motor cortex, there was a decrease of kidney volume and an increase of limb volume irrespective of changes in aortic pressure, the variable factor being the type of anesthetic. While a quantitative comparison of the results of various experiments cannot be made, it is possible that under ether the splanchnic constriction was relatively greater than the somatic dilatation so that the net result was an increase in the mean peripheral resistance and a rise in blood pressure; whereas in those carried out under Dial and ether, the somatic vasodilatation was relatively greater than the splanchnic constriction resulting in a reduction of the mean peripheral resistance and a decline of blood pressure. Such effect of different anesthetics may explain the observations of Howell and Austin (21) that a rise of pressure was obtained from the sigmoid area of the dog when morphia and curare were used and a fall when morphia and ether were administered.

On the basis of these stimulation experiments, it may be concluded

that *there are certain nervous pathways, originating in the motor cortex, excitation of which results in a redistribution of blood, decreasing the supply to the abdominal viscera and increasing that to those parts which would be made active by the simultaneous excitation of the motor efferent pathways from the same areas of the cortex.* Such excitation may presumably occur under conditions of normal voluntary activity. Since an apparently adequate circulation may be maintained in decerebrate animals (cats) in which vascular reflexes can be elicited, the question arises what essential relationship exists between the cerebral cortex and vasomotor activity in normally functioning individuals. In an attempt to clarify this problem, certain deductions from stimulation experiments are presented: 1, most of the responses to cortical stimulation are "type" responses, i.e., essentially the same irrespective of the part of the motor cortex stimulated; 2, this "type" response is usually such that it causes a shift of blood from the splanchnic to the muscular regions; but 3, this response is subject to profound alteration by factors extraneous to the site of stimulation and the character of the stimulus, i.e., the general blood pressure, the type and depth of anesthesia, the depth of the pulmonary ventilation and the state of the animal as influenced by the duration of the experiment.

Two theories of the relationship of the cortex to the vascular system may be advanced to account for these observations. The first implies that the ordinary distribution of blood is maintained primarily by reflexes operating through medullary or hypothalamic centers. According to this conception, corticofugal impulses, which may be considered afferent with regard to these subcortical centers, act to upset temporarily this balance in favor of a different blood distribution, while the lower centers reëstablish the original state after the cessation of impulses from the cortex. Whether the effect of stimulation of the cortex is a rise or fall of pressure or a constriction or dilatation of the limbs, in any case the response is mediated through the same cortifugal pathways passing to those subcortical centers, the difference in the end result being due to the various factors considered above which operate in such a manner as to modify the state of reactivity of the subcortical vascular reflex centers.

The second theory presupposes that vasomotor responses are integrated at the level of the cerebral cortex as well as at subcortical levels. The cortex may be more particularly concerned with the finer integration of somatic muscular and vasomotor activities and the adjustment of these responses to exteroceptive afferent impulses. On the basis of this theory, the various anesthetics, the depth of the respiration, etc., may alter the response to cortical stimulation not only by affecting subcortical centers but also by direct action on the cortex itself. This theory is in harmony with the view expressed by Pinkston, Bard and Rioch (22) that "several levels of the nervous system including the cortical, are necessary for normal temperature control."

Since conclusive evidence of the exact part played by the cortex in the integration of cardiovascular responses is not yet forthcoming, it is impossible to decide between the two theories presented above. Future investigation will probably reveal that the two views are not mutually exclusive, but that the correct explanation of the higher control of autonomic functions will involve a combination of them both. However, the present experiments do establish the experimental fact that excitation of efferent nervous pathways from the motor and premotor areas of the cerebral cortex results in alterations, both in direction and in extent, of the vasoconstrictor state of widely separated areas and that a mechanism thus exists by which the cortex may influence the distribution of blood in the body.

SUMMARY

1. Stimulation of the sigmoid gyrus of cats and of the cortex adjacent to the superior precentral sulcus in monkeys anesthetized with Dial-Ciba plus curare usually evokes no change or a fall of pressure, whereas with ether anesthesia the pressure usually rises.

2. When limb and renal volumes are recorded simultaneously, the limb volumes usually increase while the kidney volume diminishes during excitation of the motor and premotor areas of both cats and monkeys.

3. The alterations of volume of the limbs in response to stimulation of the motor areas of the cortex involve the muscle predominantly, since the changes persist essentially unaltered after removing the skin. They occur in the same direction in opposite extremities but in the monkey are often, though not consistently, somewhat greater in the contralateral extremities.

4. Since dilatation of the limbs occurs with a fall of blood pressure and constriction with a rise; since these alterations of limb volume are abolished by denervation of the limbs, and since they can be produced in non-curarized animals with stimuli too weak to cause muscular activity, it is concluded that alterations of blood flow in the extremities occurring as a result of excitation of the cortex are active phenomena mediated through nervous channels and not the product of epileptic seizure nor passive changes due to alteration in blood pressure.

5. In spite of the fact that the blood pressure usually rises with excitation of the motor cortex of animals under ether anesthesia and falls in animals anesthetized with Dial-Ciba, the direction of the simultaneous changes of volume of the kidney and limbs is usually the same under both anesthetics. It is suggested therefore that the rise of pressure in the former may be due to a predominance of splanchnic vasoconstriction while the fall of pressure in the latter may be due to predominance of somatic vasodilatation. However, occasionally in instances of low initial systemic blood pressure or diminution of pulmonary ventilation, reduc-

tion of limb volume instead of dilatation is induced by the stimulation. It is concluded therefore that a synergic or reciprocal relationship exists between the degree of constriction and dilatation of the arterioles in various parts of the body and that this relationship may be disturbed by excitation of the cortex. While such excitation usually alters this relationship in such manner as to favor the circulation in active muscles of the body, the distribution, extent and direction of the alteration may be affected by many factors. Therefore any conceivable variation of mean blood pressure can occur.

6. On the basis of these experiments it is concluded that a mechanism exists by which the motor and premotor areas of the cerebral cortex may influence the distribution of blood to various regions of the body and that through this mechanism the requirements for increased blood supply in muscles brought into action by the motor cortex may possibly be anticipated.

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A STUDY OF THE BLOOD SUGAR RAISING SUBSTANCE IN THE URINE OF DIABETIC AND NON-DIABETIC PATIENTS

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The following study was intended to investigate the urines of non-diabetic patients, controlled diabetics using insulin and those not requiring insulin, and uncontrolled diabetic patients, for the presence of a blood sugar raising substance and to make a survey of the several chemical properties of this substance if found.

The work of Houssay and his collaborators (1) on the pituitary and pancreas indicated the presence of a blood sugar raising substance in the secretion of the pituitary gland. Since the publication of this work, which has subsequently been repeated (2, 3, 4, 5), a similar factor has been studied in the blood and urine (6, 7). Potent extracts of the blood sugar raising principle from human urine were prepared and studied by Houssay and Biosotti (8) before 1933. The method of preparation described in this last paper was accepted for the following work, being modified only to suit the experiments undertaken.

EXPERIMENTAL. The so-called diabetogenic substance used in the experiments below was prepared from the urine of 6 male and 5 female patients: 4 controlled and 2 uncontrolled diabetic patients taking insulin, 2 controlled diabetic patients not taking insulin and 3 non-diabetic patients. In each case the product was tested on three rabbits and three dogs, which were previously starved for 24 hours.

Seventy-two hour urine specimens were collected, evaporated under suction to small volumes and then the method described by Houssay and Biosotti was followed. The final product was evaporated down to 40 cc. in each case for uniformity and injected intravenously in 2 cc. quantities. Blood samples for quantitative sugar determinations were taken before injection and at the following intervals after injection: 15 and 30 minutes, 1, 2 and 3 hours.

In comparing the blood sugar readings it is convenient to use the one obtained from the one hour blood specimen.

In the set of experiments carried out on the factor prepared from the urine of non-diabetic patients, the blood samples taken after injection of the product gave sugar readings closely resembling the one obtained

before injection. For one series of three dog experiments the average rise at the one hour specimen was 5.3 mgm. per 100 cc. of blood and in a rabbit series the variation was 9.3. In another series the difference in the case of dogs was 4.6 mgm., while in rabbits it was 12.

Results obtained by using the factor furnished by the urine of controlled diabetic patients taking insulin were unlike those observed from the products of the non-diabetic urines. The lowest average of one hour specimens given in the case of a series of three rabbit experiments was 34.3 mgm. per 100 cc. of blood, the highest being 50.6. For dogs the lowest was 39.3, while the highest was 56. For the twelve rabbit tests on the four products furnished by this group of patients the average one hour rise was 44.1 mgm. and for the twelve dogs it was 45.7.

The factor prepared from uncontrolled diabetic patients taking insulin gave results resembling very much those furnished by the factor obtained from the urines of controlled diabetic patients taking insulin. In the case of a sequence of three rabbit experiments performed here, the average sugar rise for the one hour blood samples was 62.5 mgm. per 100 cc. of blood, with 61.0 for the dogs.

The diabetogenic substance provided by the urines from diabetic patients controlled with diet only (insulin unnecessary) rendered sugar readings, for the one hour blood specimen, with an average rise of 18.75 mgm. per 100 cc. of blood, in the case of a dog sequence, whereas for rabbits it was 17.5.

In addition to the urine of non-diabetic patients as a control, a blank experiment was done using water for the preparation of the hormone. Here also the product was concentrated to 40 cc.; 2 cc. and 4 cc. quantities were injected into the same animals used for the other experiments. The average rise obtained here from the one hour blood specimens, in the instance of rabbit tests, was 6.5 mgm. per 100 cc. of blood, while in dogs the variation was 7.5.

To see 1, whether a mathematical relationship could be observed between the quantity of product injected and the degree of the blood sugar rise, and 2, whether the factor, heated to boiling, would affect the blood sugar level, the following experiment was performed. The hormone injected was prepared from one of the controlled diabetic patients taking insulin. In the first test, 2 cc. of the product were injected; the second, 4 cc.; and the third, 2 cc. after the substance had been subjected to boiling for one hour. The three tests described were performed on both a rabbit and a dog. In the case of the rabbit, the rise in the blood sugar observed from the one hour specimen was, for the first test, 34 mgm. per 100 cc. of blood, for the second, 78 mgm. and the third, 8. For the dog sequence, the rise for the first was 8, for the second, 34, and for the third, 3.

Experiments were performed to determine if the factor was a protein or absorbed with one. The protein from a 24-hour urine specimen of a controlled diabetic patient taking insulin was precipitated with ammonium sulphate, filtered and the filtrate dialyzed until a negative barium sulphate test was observed. A biuret test on this material was also negative. The preparation of the factor was then made from this protein-free urine according to the Houssay method, and the product after concentration to 40 cc. was injected intravenously into two dogs in 2 cc. quantities. Blood for sugar determinations was drawn before injection and after injection. It is inferred that the active agent is not associated with the urinary protein.

Another experiment was done to determine if kaolin was capable of removing the factor. The material remaining in the above experiment after adsorption by kaolin (5 grams of kaolin per 500 cc. of urine) when concentrated and injected in dogs did not give rise to an increase in the blood sugar.

Since the preparation of the substance by the above method in which the protein was not first separated resulted in a product which also gave a negative biuret test, this method employing whole urine was used for the remaining experiments reported in this paper.

The fact was found to be adsorbed by kaolin and charcoal and soluble in water and 60 per cent alcohol. Intravenous injection of such extracts produced definite rises of the blood sugar in the animals tested. The substance, however, was found to be insoluble in fat solvents, since no rise in the blood sugar was observed when a chloroform, ether, or carbon tetrachloride extract was injected intravenously.

The effect of boiling was then tested. Boiling for an hour completely destroyed the factor and no rise in the blood sugar occurred when this material was used.

A Berkefeld filtrate of the material at pH 5, 7 and 9 similarly gave no blood sugar rise, indicating that the factor does not pass through ultrafilters.

Discussion of results. Since the material remaining in the urine after the removal of the protein gave a blood sugar rise in the animals used, it was evident that we were not dealing with a protein reaction.

Our results are in accordance with the findings of Houssay and his collaborators; namely, that the blood sugar raising substance is easily adsorbed, soluble in water and in 60 per cent alcohol and insoluble in fat solvents. It does not pass through ultrafilters and boiling destroys its activity. These results do not agree entirely with those found by Anselmino and Hoffman in their studies of the factor contained in the blood serum or extracted from the anterior lobe of the hypophysis. These workers found that the substance will pass through a Berkefeld filter at pH

5.3 with only a slight loss of activity, and at pH 9.4 it will pass through in traces; and that its effectivity is only partly lost by boiling. It would be desirable to know if the substances obtained from the hypophysis, from the blood serum and from the urine are the same.

One may observe from the experiments performed on the factor furnished by normal urine, that it is present in non-diabetic urine, if at all, in small quantities, for the blood sugar rise is within the possible experimental error. From those experiments performed on the factor obtained from the urine of controlled diabetic patients taking insulin, one may conclude that it is present in considerable quantity. The factor prepared from uncontrolled diabetic patients taking insulin, gave readings very much like those obtained by the substance of the preceding group. In other words, a reasonable blood sugar rise above the experimental error may be seen. The blood sugar rise given by the factor prepared from controlled diabetic patients not taking insulin is not as great as that given by the factor prepared from controlled and uncontrolled diabetic patients taking insulin, in whom the diabetes was obviously more severe. However, the rise is greater than the expected experimental error and hence the substance may be considered present in at least some quantity.

CONCLUSIONS

1. A blood sugar raising substance is found in the urines of severe diabetic patients, and may be found to a lesser degree in the urines of mild diabetic patients; and in non-diabetic urine it is found, if at all, in small quantities.
2. The factor, in all probability, is not a protein.
3. It is easily adsorbed by kaolin and charcoal.
4. It is soluble in water and 60 per cent alcohol.
5. It is insoluble in fat solvents.
6. It does not pass through a Berkefeld filter.
7. It is destroyed by boiling.

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POSITIVE FRIEDMAN TESTS IN THE PREGNANT RHESUS MONKEY, MACACA MULATTA

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The Friedman pregnancy test, which has proven so reliable for the human, has almost uniformly failed when tried on other animals, only the chimpanzee having given positive reactions. The macaque has heretofore yielded only negative results, Snyder and Wislocki (1) having tested the urine from this monkey from the end of the first month of pregnancy to parturition without ever securing a positive test.

Because of an idea that in the monkey the excretion of prolan in the urine might be a transitory process, occurring only during the establishment of the placenta, I have recently made a series of tests on pregnant macaques in the Carnegie colony, starting the testing as early as possible during pregnancy. These tests show that the pregnant monkey gives a perfectly typical, positive Friedman reaction for a period of about a week soon after implantation. The failure of other investigators to induce ovulation in rabbits by the injection of monkey pregnancy urine is undoubtedly due to their having started the tests too late to detect the transitory presence of the urinary prolan.

Figure 1 gives the data for all the relevant cases. In several series of tests the monkeys eventually proved to be non-pregnant; these tests were uniformly negative and have been omitted from the table. It was not possible to secure a larger series of tests, as most of the pregnancies in the colony were terminated by operation, prior to the onset of the prolan-excreting period, for the purpose of securing early embryos for the embryological studies. The amount of urine injected varied considerably, as shown in the figure. It was thought at first that the negative results of other workers might have been due to a dilution of the hormone as compared to human urine of pregnancy, and an attempt was accordingly made to secure at least 15 cc. of urine for each injection. The last monkey tested, no. 299, showed however that 5 cc. is sufficient to give a positive reaction during the prolan-excreting period.

It will be seen that positive reactions were secured only during the nineteenth to twenty-fifth days following ovulation and conception. Sev-

eral pregnant individuals tested before the fifteenth day (omitted from the figure) were negative, and monkey 303 gave a negative reaction on the seventeenth day. On the other hand, the prolan may disappear somewhat earlier than the twenty-fifth day, as monkey 76 had already become negative when she was first tested on day 24. This extremely transitory excretion of the urinary prolan compares quite closely with the curve of its output in the pregnant woman, as found in the recent study of Browne and Venning (2). In the woman, however, while there is a tremendous drop in the number of units of prolan excreted, and while this drop occurs at a time (about the eighth post-menstrual week of gestation) somewhat comparable to that in the monkey, the base level of prolan output remains high enough throughout the remainder of pregnancy so that 5 cc. of unconcentrated urine will always yield a positive test. In the macaque, on the other hand, the output drops to such a low level that 20 cc. of urine,

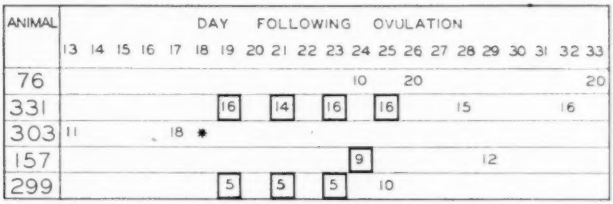

Fig. 1. The numbers are the number of cubic centimeters of urine injected into the test rabbits. Those in the leaded squares represent positive tests, the others gave negative results.

* Embryo removed at operation on day 18.

after the twenty-fifth day, does not contain even a single rabbit unit of prolan.

The situation in the apes is not yet clear. Schultz and Snyder (3) obtained positive reactions from the urine of pregnant chimpanzees at 95 and 100 days, post-menstrual computation, and negative ones from day 160 on. Earlier and more closely seriated observations for this species are highly desirable. It appears, on the basis of our present knowledge, as if we might be dealing here with a phylogenetic series in the evolution of a physiological character. The excretion of detectable quantities of prolan, which is such a transitory phenomenon in the catarrhine monkey, becomes a three-month-long process in the ape, while in the human it has developed into a physiological characteristic of such stability that it persists from its first appearance, shortly after implantation, throughout gestation, and disappears only after parturition. It will be of the greatest interest

to learn if any traces of this process are to be detected in the new-world monkeys, a possibility however which appears extremely remote.

It is a pleasure to acknowledge here my indebtedness to Dr. Carl G. Hartman, for the use of his monkey colony, and to Dr. J. E. Markee for his assistance in the tests.

SUMMARY

The pregnant macaque was found to excrete the rabbit-ovulating prolan in the urine between the nineteenth and the twenty-fifth day of pregnancy, none after that.

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THE DIRECT DETERMINATION OF THE RENAL BLOOD FLOW AND RENAL OXYGEN CONSUMPTION OF THE UNANESTHETIZED DOG[1]

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The rate of renal blood flow has been determined by a number of observers who have worked with anesthetized animals. The results of such investigations have been summarized by Van Slyke et al. (1934). These authors, using unanesthetized dogs with a kidney explanted by the technique of Rhoads (1934), obtained blood from the renal vein and calculated the renal blood flow from the difference between the urea contents of arterial and renal venous blood and the urine urea excretion over a given time. From the blood flow and the oxygen lost by a unit of blood in passing through the kidney the renal oxygen consumption was computed.

Other measurements on unanesthetized animals include those of Medes and Herrick (1933), Herrick, Essex, Baldes and Mann (1936), and Handovsky and Samann (1935) who used the thermo-electric stromuhr of Rein (1928) in making postoperative measurements of renal flow. The procedure to be described is the only one, so far as we are aware, permitting the direct determination of the blood flow through the kidney of the unanesthetized animal. It is the purpose of this paper to present the results obtained by this method and to compare these results with some obtained on the same animals by an indirect method modified from that employed by Van Slyke and his co-workers.

METHOD. Dogs were used. Their diet consisted of a standard kennel ration supplemented by meat three times each week. Food was withheld the evening preceding the experiment. The animals were trained to lie quietly upon the table during the procedures and gave no evidence of serious discomfort. They would often eat, fight or play immediately following completion of the studies.

They were prepared for the determination by a preliminary operation. At this time the right lumbar vein was ligated just distal to its entrance into the adrenal gland; the left adrenal vein, the spermatic (or ovarian)

[1] Aided by a grant from the Division of Medical Sciences of the Rockefeller Foundation.

veins and all other visible accessory veins a few centimeters above and below the renal veins were ligated so that only blood from the kidneys plus a very small amount from the remaining adrenal vein and from the ureteral veins entered the vena cava in this region. The exact position of the renal veins was noted and located for the future experiment by a small incision made on the skin of the anterior abdominal wall. In some instances one of the kidneys was removed at this time; in others the preparatory operation was performed on dogs which previously had had a kidney explanted by the technique of Rhoads, and the opposite kidney excised. In the remaining instances both kidneys were left intact. Determinations of blood flow were not made until the animals had fully recovered from the preparatory procedure.

The principle of the method of flow measurement consists of introducing a modified Morawitz cannula into the inferior vena cava via an external jugular vein, a route which has been used by Harrison, Friedman and Resnik (1936) in cannulating and measuring the blood flow through the coronary sinus of morphinized dogs. The details of the construction of the cannula and its application to the determination of the blood flow through the liver have been described by Blalock and Mason (1936). The results of some experiments on determining the renal flow by this procedure have been the subject of a preliminary report (Mason, Blalock and Harrison, 1936).

In applying the method to the determination of renal blood flow, the cannula was placed so that the position of the distal balloon in the inferior vena cava was about two centimeters below the left renal vein, thus fixing the position of the proximal balloon about the same distance above the right renal vein. The exact location of the cannula in the vena cava was determined by reference to the scar of the little incision made as a marker on the skin of the anterior abdominal wall directly over one of the renal vein orifices at the preliminary operation. This procedure was found to be just as effective and considerably more convenient than localization of the cannula by fluoroscopy as employed by Blalock and Mason in determining hepatic blood flow. As soon as the balloons were inflated about 30 cc. of blood were allowed to escape before starting to note the time required for the outflow of 50 cc. into a graduated cylinder. Other details of the procedure such as the level of the outflow tube, the order of inflation of the balloons, the amount of heparin employed, etc., have been described by Blalock and Mason (1936).

After satisfactory measurements of the flow were obtained, samples of blood for analyses were taken from the femoral artery and from the neck of the cannula while the blood was flowing, or from the side-arm of rubber tubing at the completion of a measurement. The samples were taken simultaneously or as nearly so as possible. Usually the measurements of

the flow were repeated after a brief rest period, and another set of samples was taken. Upon completion of the experiment the cannula was withdrawn, ligatures were placed on the jugular veins, and the neck incisions closed. By employing asepsis it is usually possible to perform at least four experiments on each animal before the remnants of the external jugular vein become inaccessible for cannulation.

In experiments in which the renal blood flow was also determined by an indirect procedure modified (vide infra) from that of Van Slyke et al. (1934), urine collections were made by catheterization. The bladder was washed several times with warm physiological saline solution prior to starting the period. At the conclusion of the period the bladder was again washed repeatedly, the period ending with the last washing. The urea contents of the final washings were determined separately as a check on the effectiveness of the procedure. The volume of urine excreted was calculated in the manner described by Rhoads, Alving, Hiller and Van Slyke (1934).

The following analytical procedures were employed in connection with these experiments. *Oxygen contents* were determined manometrically in 2 cc. samples of blood (Peters and Van Slyke, 1932, p. 321). Oxygen capacities were similarly determined after saturation of the blood at room temperature with atmospheric air. The correction employed in this determination for dissolved oxygen was 0.6 volume per cent (Sendroy, Dillon, and Van Slyke, 1934). *Whole blood urea* was determined by the gasometric urease method (Peters and Van Slyke, 1932, p. 373) in 1 cc. samples of blood with all manometer readings made at the 2.0 cc. mark. *Urine urea* was determined in some instances by the gasometric urease method (Peters and Van Slyke, 1932, p. 361) and in most instances by the hypobromite method (Peters and Van Slyke, 1932, p. 380) with the modifications that the removal of ammonia by treatment with permutit was omitted (Van Slyke, Page, Hiller, and Kirk, 1935) and the improved reagent of Van Slyke and Kugel (1933) was used. It was found that the two procedures yielded practically identical values.

Results. The results obtained from dogs with two kidneys are given in table 1. In some instances two sets of measurements of the renal blood flow were made and two sets of blood samples were obtained in the course of one experiment. In other instances the second set of measurements and samples were omitted. Each recorded value of the rate of flow is the average of several consecutive measurements.

In three experiments the flow was simultaneously estimated by the "indirect" method. The procedure of Van Slyke et al. was modified in several respects in these instances. The renal venous blood was taken from the cannula rather than by renal vein puncture. In addition, the blood samples were taken in the middle of a single period of collection of urine

of about sixty minutes' duration and the observed arterio-renal venous urea difference was assumed to be the average of that for the entire period. The rate of urine flow was above the augmentation limit as a result of fluid given in administration of the heparin prior to the experiment.

TABLE 1

Renal blood flow and oxygen consumption of dogs with two kidneys

DOG	DATE	WEIGHT	OXYGEN CONTENTS			RENAL BLOOD FLOW			RENAL OXYGEN CONSUMPTION	
			Arterial	Renal venous	A-V O_2	Direct method		Total "indirect" method*		
						Total	Per kgm. dog		Total	Per kgm. dog
		kgm.	*vols. per cent*	*vols. per cent*	*vols. per cent*	*cc. per minute*	*cc. per minute*	*cc. per minute*	*cc. per minute*	*cc. per minute*
1 ♂	6/19	15.80	17.32	14.39	2.93	275	17.4		8.06	0.51
			17.17	14.28	2.89	309	19.6		8.93	0.56
1 ♂	7/17	14.75	19.90	17.64	2.26	236	16.0		5.34	0.36
			19.90	17.45	2.45	281	19.0		6.89	0.47
2 ♂	6/24	12.40	16.06	12.57	3.49	362	29.2		12.63	1.02
			16.78	12.98	3.80	370	29.8		14.06	1.13
2 ♂	7/21	12.30	18.40	15.24	3.16	303	24.6		9.58	0.78
3 ♂	6/27	21.50	21.19	18.20	2.99	476	22.1		14.23	0.66
			21.55	18.32	3.23	509	23.7		16.44	0.77
3 ♂	7/22	23.10	17.24	14.63	2.61	435	18.8		11.35	0.49
			17.28	14.55	2.73	455	19.7		12.42	0.54
4 ♀	6/22	18.00	15.95	13.76	2.19	319	17.7		6.99	0.39
4 ♀	7/18	17.66	19.06	17.01	2.05	330	18.7		6.77	0.38
5 ♂	6/20	15.00	15.83	13.00	2.83	326	21.7		9.23	0.62
			15.97	13.42	2.55	361	24.1		9.20	0.61
6 ♂	6/23	12.80				286	22.3			
7 ♀	4/21	16.10	20.30	17.53	2.77	261	16.2		7.23	0.45
8 ♂	3/30	13.10	17.75	15.28	2.47	290	22.1	251	7.16	0.55
9 ♂	2/27	17.95	15.10	12.52	2.58	476	26.5	493	12.27	0.68
10 ♀	4/2	14.80	20.78	16.99	3.79	266	18.0		10.07	0.68
11 ♀	4/27	18.00	19.85	15.94	3.91	275	15.3	316	10.75	0.60
12 ♂	3/25	13.10	22.12	18.52	3.60	303	23.1		10.90	0.83

* By analysis of renal venous blood from the cannula.

In calculating the oxygen consumption of the kidney the correction employed by Van Slyke and his co-workers for differences in oxygen capacity between arterial and renal venous blood has not been made. In some of the earlier experiments we found the renal vein oxygen capacity to be as much as 1.5 volumes per cent *below* the corresponding arterial capacity; however, estimation of water contents failed to show that this was associated with a shift of fluid from tissues to blood during the

passage of the latter through the kidney. It was then observed that *immediate* saturation and analysis of the blood usually reduced the difference to the range of gasometric accuracy and it was concluded that the shifts of water that actually did occur could be neglected without serious error.[2, 3]

The results obtained from dogs with one explanted kidney are summarized in table 2. One or more "indirect" renal blood flow determinations were made on each of these animals prior to the direct determination at which time both the direct and "indirect" (calculated from analyses of blood obtained by puncture of the renal vein) flows were determined simultaneously. The curious phenomenon noted by Van Slyke et al. of

TABLE 2

Renal blood flow of dogs with one kidney (explanted) determined by the direct and "indirect" methods alone and simultaneously

	RENAL BLOOD FLOW									
	Dog 13	Dog 14	Dog 15	Dog 16	Dog 17	Dog 18	Dog 19	Dog 20	Dog 21	Dog 22
	cc. per minute	*cc. per minute*	*cc. per minute*	*cc. per minute*	*cc. per minute*	*cc. per minute*	*cc. per minute*	*cc. per minute*	*cc. per minute*	*cc. per minute*
By "indirect" method	107	226	194	198 155 162 180	69 78 73	96 140	243 170	114 114	341	
By direct method	300 294		259					121		
By "indirect" and direct methods simultaneously (italics signify "indirect" flows)		187, *149* 208, *327** 288,† *564*	280, *206*	158, *437*	141, *90* 167,† *90*	270, *179* 358,† *177*	319, *518*	140, *190*	316, *185* 301, *415*	303, *271*

* Venous blood taken from cannula instead of by renal vein puncture.
† Considerable degree of anemia.

the renal vein urea concentration occasionally equaling or exceeding the arterial concentration was encountered several times, for the most part, unfortunately, in those experiments in which both the direct and "indirect" flows were being determined. In these instances, of course, the "indirect" flow could not be calculated. This difficulty was experienced

[2] In calculating the oxygen consumption of the liver, Blalock and Mason retained the correction as much larger degrees of fluid exchange between tissues and blood might be expected to take place in this organ.

[3] Significant oxygen capacity differences occurred more frequently when the venous sample was obtained by puncture of the renal vein of dogs with explanted kidneys than when the sample was obtained by the cannula.

only in dogs from which the renal venous blood sample was taken by puncture of the renal vein.

In the course of developing the cannulation procedure finally used, certain modifications were tried. Two of these at one time seemed promising and several experiments were performed with each.

The first consisted of employing external occlusion of the vena cava around the cannula proximal to the right renal vein, the distal tip of the cannula resting against a previously inserted ligature with which the vena cava was occluded about 2 cm. below the left renal vein orifice. At the time the vena cava (and accessory vessels) were ligated, a rubber cuff designed to expand principally inwardly was sewed into position around the vena cava above the right renal vein. A thin rubber tube led from the cuff out through the anterior abdominal wall. Inflation of the cuff could be accomplished with a syringe using water or air, and with the cannula (unequipped with balloons) in position the return of blood by this point was fairly effectively blocked. This procedure was abandoned because of the high incidence of infection, the frequent failure to achieve complete occlusion, and because of the tendency for the development of significant collateral vessels which emptied into the vena cava in the neighborhood of the renal veins. In spite of these difficulties, several satisfactory measurements of the renal blood flow were obtained.

The second modification consisted of substituting an internally expanding balloon attached to the cannula for the externally occluding cuff, the cava being ligated below the renal vein. As collaterals still tended to develop, this technique was also discarded; however, several satisfactory measurements were made.

It should be noted that the error introduced by collateral venous blood flow into the vena cava affects the accuracy of the oxygen consumption calculations much more than it affects the total rate of flow because the oxygen unsaturation of such blood is two to three times that of renal venous blood.

The results of the determinations made by these modifications are summarized in table 3. Calculation of the renal oxygen consumption has been omitted as in these experiments there was no doubt that a small proportion (on the order of 5 per cent) of the blood delivered by the cannula was non-renal in origin. In several of these experiments simultaneous "indirect" determinations were made, the renal sample being obtained from the cannula.

Discussion. *Renal blood flow.* In a series of twenty-two observations on twelve dogs (table 1) the rate of renal blood flow was found to be from 16.0 cc. to 29.8 cc. per minute per kilogram body weight with an average flow of 21.1 cc. The rates obtained by modifications of the method were not materially different. These values are considerably larger than those

obtained by workers using anesthetized animals, and are of the same order as those we calculated from the data of Van Slyke et al. who found the

TABLE 3

Renal blood flow of unilateral nephrectomized dogs and normal dogs determined by modifications of the direct method

DOG	DATE	WEIGHT	MODIFICATION EMPLOYED IN DETERMING FLOW	RENAL BLOOD FLOW		
				PER DOG	Per kgm. dog	By simultaneous "indirect" method*
		kgm.		*cc. per minute*	*cc. per minute*	*cc. per minute*
23 ♀ (one kidney)	12/2	16.40	External occlusion; ligation of vena cava	265	16.2	229
24 ♂ (one kidney)	11/30	13.20	External occlusion; ligation of vena cava	265	20.1	273
	12/11	13.00		275	21.2	212
25 ♀ (one kidney)	1/18	14.50	External occlusion; ligation of vena cava	141	9.8	
	1/21	15.20		167	11.0	
26 ♀ (one kidney)	11/27	14.70	External occlusion; ligation of vena cava	259	17.6	373
	12/4	14.70		288	19.6	
27 ♂ (one kidney)	12/21	11.27	External occlusion; ligation of vena cava	189	16.8	
28 ♀ (one kidney)	12/6	16.35	External occlusion; ligation of vena cava	233	14.2	204
29 ♀ (two kidneys)	12/9	17.00	External occlusion; ligation of vena cava	309	18.2	251
	12/13	17.00		323	19.0	
30 ♀ (two kidneys)	2/25	14.60	One internally expanding balloon; ligation of vena cava	268	18.3	
	2/29	15.00		333	22.2	
31 ♂ (two kidneys)	2/24	11.00	One internally expanding balloon; ligation of vena cava	216	19.6	195
	2/28	11.00		206	18.7	186
32 ♂ (two kidneys)	2/22	17.60	One internally expanding balloon; ligation of vena cava	406	23.0	
	2/26	17.60		429	24.4	860

* By analysis of renal venous blood obtained from the cannula.

renal blood flow of their dogs with two kidneys to average 19.3 cc. per minute per kilo body weight with their "indirect" method. Nine obser-

vations on six dogs following unilateral nephrectomy (table 3) also agree with their findings that the renal blood flow under these circumstances is diminished approximately 30 per cent.

Renal oxygen consumption. The values obtained for renal oxygen consumption are nearly three times as great as those found by Barcroft and Brodie (1904) in experiments in which anesthesia was employed. The average value for the oxygen consumption *per kidney* of dogs with two kidneys was found to be 5.0 cc. per minute (table 1); the corresponding average calculated by Van Slyke et al. was 3.1 cc. per minute. Three factors participate in accounting for this difference. 1. These workers employed somewhat smaller dogs than were used in our experiments. 2. Small collateral vessels contribute a slight amount of more highly reduced non-renal blood to the total volume of blood passing through the cannula in our method, thus slightly increasing the calculated value of the renal oxygen consumption.[4] 3. The correction applied by Van Slyke et al. for the usually observed apparent decrease in hemoglobin content of renal venous blood considerably diminishes the calculated value of the oxygen consumption, and this correction has been omitted in our calculations (vide supra). The latter factor accounts for much of the disparity between the values obtained by the two methods, and if, as we believe, the extreme differences in oxygen capacities of renal venous and arterial bloods are largely artefacts, the true renal oxygen consumption probably lies between the values obtained by the above mentioned workers and ourselves.[5]

The comparison of the direct and "indirect" methods. Thirteen determinations of the renal blood flow, by direct and "indirect" methods simultaneously, were made in which the renal venous blood samples were obtained from the cannula (tables 1 and 3). Similar determinations were also made in thirteen instances in which the procedure was the same except that the renal venous blood was drawn by venipuncture of an explanted kidney (table 2). In the former series in eight out of the thirteen determi-

[4] At the conclusion of some experiments the animals were anesthetized, the abdomen opened, and the renal veins ligated at their point of entry into the vena cava. The vena cava was then ligated above and below the renal veins at points which would be completely occluded by the cannula balloons during a determination of the renal blood flow, cannulated between these ligatures and the rate of flow of blood noted. This measured the non-renal collateral flow which was found to be on the order of 5 cc. per minute, or about one to four per cent of the measured renal blood flow. In the absence of anesthesia it probably would have been somewhat greater.

[5] We have observed occasionally significant differences in oxygen capacity between arterial and renal venous blood, when the latter was obtained by puncture of the renal vein of explanted kidneys, in spite of the fact that the bloods were immediately saturated and analyzed. This did not occur when the renal venous blood was obtained from the cannula. We have no explanation for this.

nations, the direct and "indirect" flows agreed within twenty per cent, and only two differed by more than thirty per cent. In the latter series the agreement was poor. In only three observations out of thirteen did the direct and "indirect" flows agree within twenty per cent and nine differed by more than thirty per cent. In most instances in which one or more "indirect" flows were determined on dogs with an explanted kidney before direct measurements were made, the calculated "indirect" flow rates were lower than the subsequently directly determined values (table 2).

It seems likely that these differences are due to the fact that the "indirect" procedure of Van Slyke et al. was modified in several respects in our experiments (vide supra). That the "indirect" procedure as used by these investigators yields rates of flow comparable with those obtained by our direct method is indicated by the close agreement between the average rates of flow per kilo dog.

Certain features of the direct method make it a useful investigative procedure. The functional activities of both kidneys may be studied without moving either from its normal anatomical position. Large amounts of renal venous blood may be obtained readily without employing renal venipuncture, a procedure which seems, occasionally, to affect temporarily certain phases of renal function. In addition simultaneous studies may be made of functions of other organs, for example, that of the liver (Blalock and Mason, 1936). This may be of importance in instances in which both these organs are concerned in different phases of the physiological process under scrutiny.

SUMMARY

1. The renal blood flow and oxygen consumption of unanesthetized dogs has been determined by a cannulation procedure.

2. The renal blood flow averaged 21.1 cc. per minute per kilogram body weight. Unilateral nephrectomy diminished the flow approximately 30 per cent.

3. The renal oxygen consumption averaged 5.0 cc. per minute per kidney.

4. The results of the determinations of renal blood flow are compared with those obtained by the employment of an indirect method.

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EFFECTS OF CORTICO-ADRENAL EXTRACT ON GROWTH AND SEXUAL ACTIVITIES[1]

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Many attempts have been made experimentally to produce changes similar to those observed in cases of tumors of the adrenal cortex. In most instances these tumors produce profound changes in sexual characteristics (reviews of Krabbe, 1921; Glynn, 1921; and Meyer and Frumess, 1931). The results with transplantations, with feeding of adrenal tissue, and with extracts of the adrenal cortex have been conflicting. Among the more recent experiments Corey and Britton (1931) stimulated the sex organs of normal young rats with corticoadrenal extract. With the exception of the observations of Atwell (1932), Migliavacca (1933), Casida and Hellbaum (1934), and Allen and Bourne (1936), the evidence fails to confirm the sexual precocity reported by Corey and Britton.

Hypoactivity of the adrenal cortex often interferes with sexual functions. Amenorrhea, atrophy of the testes and ovary, absence of libido, and impotence may occur in patients suffering from Addison's disease (Rowntree and Snell, 1931). Structural as well as functional changes in the gonads have been reported to follow adrenalectomy (Freed, Brownfield and Evans, 1931; Martin, 1932; Firor and Grollman, 1933). Furthermore, the ovarian cycle, pregnancy, and lactation are affected (Corey and Britton, 1934; Britton and Kline, 1934; Hartman, 1933).

1. *Sexual maturity of immature rats treated with cortico-adrenal extract. Methods.* Litters containing from five to ten young were selected from the laboratory stock of albino rats. The exact age of the young was known in all instances and no litters that showed any great variation in the weight of the individuals were used. The young rats were divided into control and experimental groups according to size and sex, and were left in the cage with their mother until the termination of the experiment.

The experimental rats received daily doses of cortico-adrenal extract. This extract was prepared according to a slight modification of the method

[1] The data in this paper are taken from a dissertation presented to the Graduate Faculty of the University of Virginia in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

of Swingle and Pfiffner (Britton and Silvette, 1931). The dosage varied for the twenty-six litters from 0.25 cc. to 5 cc. All injections were made intraperitoneally. The controls in most cases were given an injection of

TABLE 1

The effect of cortico-adrenal extract on the sex organs of young rats

LITTER NUMBER	AGE OF RATS WHEN KILLED	LENGTH OF EXPERIMENT	EXTRACT DOSAGE	NUMBER OF RATS IN LITTER	WEIGHT DIFFERENCES EXPERIMENTALS-CONTROLS		
					Ovaries	Uteri	Testes
	days	*days*	*cc.*		*per cent*	*per cent*	*per cent*
1	30	14	½	7	+13.2	+63.1	−14.5
2	28	14	1	6	+44.4	+11.2	−9.6
3	32	14	¼	5	+16.9	+64.9	−31.9
4	36	21	¼	6	+13.4	+22.6	−30.6
5	35	21	½	6	+82.5	+39.5	−33.1
6	33	14	1	6	+37.7	+11.4	−49.3
7	30	7	2	7	+27.6	+12.8	−41.2
8	36	21	2	6			−6.5
9	31	14	1	6	+6.8	+16.3	
10	31	14	2	6	+17.5	+26.3	−16.8
11	28	14	1	5	+3.7	+22.2	−26.1
12	36	16	*1 per 10 gm. rat*	7	+21.6	+25.0	−21.2
13	31	14	2	6	+20.4	+6.3	−17.1
14	30	18	1	5	+5.4	+10.3	−49.7
15	32	16	1	5	+20.6	+31.6	
16	32	14	1	6	+6.0	+14.0	−15.7
17	42	24	1	8	+25.0	+5.4	−13.9
18	42	26	1	6	+14.0	+8.3	−3.4
19	30	14	1	10	+29.0	+12.2	−16.8
20	28	14	1	8	+22.8	+6.7	−6.3
21	28	14	1	8	+48.7	+13.4	−1.0
22	30	14	1–2	8	+22.5	+20.5	−9.5
23	30	15	2–4	7	+30.8	+48.5	−24.0
24	32	15	2–5	6	+20.8	+32.6	−27.1
25	44	28	2–5	7	+21.4	+11.7	−5.9
26	40	28	2–5	8	+20.6	+18.6	−48.2
Means					+23.7 ±2.23	+22.3 ±2.23	−21.6 ±2.03

normal saline equal to the dose of cortico-adrenal extract given the experimentals.

Great care was taken in dissecting and weighing. After all fat had been removed, the tissues were placed in covered aluminum pans and weighed immediately. Microscopic preparations were made of the ovaries, uteri, and testes from ten litters (table 2). Two or more slides were made for

each of these organs in both the experimental and control groups. All organs were fixed in formalin and cleared in xylol. Measurements of follicular, uterine, testicular, and tubular diameters were taken by means of a Filar micrometer.

The effect of cortico-adrenal extract on ovarian growth. The average ovarian weight in every litter was in favor of the cortico-adrenal extract-treated rats (table 1). The difference varied from 3.7 per cent for litter 11 to 82.5 per cent for litter 5. Hypertrophy of the ovaries was most marked in litters 2, 5, 6, 19, 21 and 23, with a percentage increase in the extract-treated animals of 44, 83, 38, 49, and 31 per cent respectively. The ovaries in seventeen out of the twenty-five litters showed an advantage in favor of the experimentals of 20 per cent or greater. The mean difference

TABLE 2

The effect of cortico-adrenal extract on the diameters of certain sex organs of young rats

LITTER NUMBER	NUMBER OF RATS	FOLLICULAR DIAMETERS		UTERINE DIAMETERS		TESTICULAR DIAMETERS		TUBULAR DIAMETERS	
		Experiment	Control	Experimental	Control	Experiment	Control	Experimental	Control
		mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
14	5	0.45	0.36	0.71	0.58	3.61	4.51	0.14	0.16
15	5	0.36	0.29	0.71	0.48				
17	8	0.38	0.34	0.77	0.68	2.93	3.88	0.13	0.15
19	10	0.40	0.35	0.66	0.58	2.89	3.68	0.12	0.12
21	8	0.35	0.34	0.70	0.55	2.69	3.30	0.09	0.12
22	8	0.37	0.30	0.65	0.51	3.25	3.52	0.13	0.14
23	7	0.33	0.29	0.80	0.56	2.15	3.44	0.08	0.11
24	6	0.35	0.28	0.63	0.43	2.79	3.73	0.11	0.12
25	7	0.41	0.32	1.12	0.78	5.44	5.69	0.16	0.21
26	8	0.36	0.28	0.78	0.69	3.68	6.24	0.15	0.18
Means.....		0.38 ±0.008	0.32 ±0.007	0.75 ±0.030	0.58 ±0.023	3.27 ±0.211	4.22 ±0.237	0.12 ±0.006	0.15 ±0.008

for the experimental group was + 23.7 ± 2.23 per cent. Moreover, only five extract-treated animals, or less than 10 per cent of the total, had ovaries which weighed less than their litter-mate controls.

Corresponding with the increased ovarian weights of the extract-treated animals were increased follicular diameters (table 2). The difference between the mean values for the experimentals and the controls is +0.06 mm. and the probable error is ± 0.010 mm. These figures are perhaps not notably significant. It was evident, however, that in every litter there had been some stimulation of follicular growth.

Large cystic follicles were observed in three extract-treated rats. Two of these animals were in the same litter and both litters had received large doses of extract. No ovarian abnormalities were noted in the control

animals. Clinically, ovarian cysts have been observed in many cases showing the typical "adreno-genital syndrome" (Broster and Vines, 1934).

Contrary to the findings of Corey and Britton, no corpora lutea were present in any of the extract-treated female rats. Evidence of precocious maturation of the ovaries following the injection of cortico-adrenal extract is, therefore, based on the increased ovarian size and on greater follicular diameters. Many enlarged follicles were seen, especially in litters 25 and 26.

The effect of cortico-adrenal extract on uterine growth. Hypertrophy of the uterus in many of the rats treated with cortico-adrenal extract was clearly detectable by eye. In every case the average uterine weight of the experimental animal was above that of the controls (table 1). Profound differences were shown in litters 1, 3, and 23, with gains of 63, 65, and 49 per cent respectively. Eleven litters out of the twenty-five showed uterine hypertrophy to the extent of 20 per cent or greater. It is significant that only three animals, or about 5 per cent, showed uteri which were smaller than their litter-mate controls. The mean hypertrophy for the twenty-five litters was 22.3 ± 2.23 per cent (table 1).

In histological section, the uterine diameters of the extract-treated animals were much larger than the controls (table 2). This fact was apparent to the eye. There is a difference in the mean value for the experimentals and the controls of 0.17 mm. with a probable error of the difference of $\pm$ 0.038 mm. The endometrial tissues showed marked hypertrophy, and the sloughing of cornified cells in some instances was indicative of approaching maturation. The lumen also was larger and much corrugated.

The effect of cortico-adrenal extract on testicular size. While cortico-adrenal extract produced a definite stimulation of growth in the sex organs of female rats, an opposite effect on the gonads of male animals was found to be the rule. The difference in size of the testes in treated and untreated animals was quite marked. The testes of the experimentals were in many cases of a "pea" size and those of the controls of a small "marble" size; that is, the control testes were nearly twice the size of the experimental animals. Testicular control weights were, with but a single exception, greater than those of the experimental rats (table 1). One experimental animal in litter 8 had a testicular weight greater than its litter-mate controls, the other two animals of this litter being in accord with the general findings for the whole series. In three litters 6, 14, and 26, the testes of the controls were approximately twice as heavy as the experimentals. The mean for the whole series shows an inhibition of testicular growth for the injected animals of 21.6 ± 2.03 per cent (table 1). The measurement of the testicular and tubular diameters corresponds to the weights of the testes (table 2). The mean testicular diameters are 3.27 ± 0.211 mm.

for the experimentals and 4.22 ± 0.237 mm. for the controls. Thus there is a difference in favor of the controls of 0.95 mm. The probable error of this difference is ± 0.317 mm. The means for the tubular diameters were 0.12 ± 0.006 mm. for the experimental animals and 0.15 ± 0.008 mm. for the control animals. The difference of 0.03 mm. has a probable error of ± 0.010 mm.

Because of the variation of potency of the many batches of cortico-adrenal extract used, it is impossible to make any general statement con-

TABLE 3

The effect of cortico-adrenal extract on growth

SERIES	LENGTH OF EXPERIMENT		SEX	NUMBER OF RATS	DAILY DOSAGE	MEAN WEIGHT GAIN
	days				*cc.*	*gms.*
1	44	Experiment	F	10	1-2	71.9±1.52
			M	10	1-2	72.1±2.52
		Control	F	10		57.3±2.41
			M	10		67.0±1.79
2	42	Experiment	F	7	1	102.0±4.42
			M	7	1	121.6±2.88
		Control	F	6		88.0±3.04
			M	5		121.0±2.82
3	21	Experiment	F	6	1	41.8±1.71
			M	5	1	50.2±2.81
		Control	F	5		34.0±1.47
			M	6		45.7±1.79
4	28	Experiment	F	5	2	61.4±1.51
			M	5	2	70.2±2.34
		Control	F	5		46.4±1.73
			M	7		63.1±2.41

cerning the effect of daily dosage or of the length of the experiments on the differences between treated and untreated rats. The few cases which tend to show pronounced increase in size of ovaries and uteri, and decrease in size of testes from large doses and extended treatment are in the minority, and it is possible that the same animals likewise received extracts of greater potency.

2. *Growth of normal immature rats treated with cortico-adrenal extract. Methods.* Four series of young healthy rats were selected from our albino stock. The first series of twenty injected and twenty control

animals were divided into control and experimental groups according to size and sex. In the other three series the controls were litter-mates of the experimentals. Initial weights of all animals were obtained and each animal was subsequently weighed on every second or fourth morning between nine and ten o'clock. The length of the experiments and the extract dosages varied as shown in table 3. Fourteen rats of the first experimental group received one cubic centimeter per day and six received two cubic centimeters per day. In the first, second, and fourth series, the cages containing the experimental and control animals were placed side by side. All animals received the same ration of grain, calf meal, milk, and occasionally green vegetables. The experimental and control animals for the third series were kept in the same cage and conditions, therefore, were identical. All the cortico-adrenal extract used in the experiment was made according to the Swingle-Pfiffner method with the slight modification of Britton and Silvette (1931). Although this extract is made by a certain standard it varies in potency from one batch to another; this factor has some bearing on the results given below. The extract was given intraperitoneally and usually at the same time each day. The control animals in the third and fourth series received equivalent amounts of normal saline.

Sex differences in growth. Table 3 shows that there was a mean weight gain for the ten injected male rats of 72.1 ± 2.52 grams and for the ten injected female rats of 71.9 ± 1.52 grams. This compares with a mean weight gain for the control males of 67.0 ± 1.79 grams and for the control females of 57.3 ± 2.41 grams. There was, therefore, a difference of only 5.1 grams in the growth increment for the injected male animals, while there was a difference of 18.3 grams in the growth increment of the injected female animals. The probable errors for these differences are ± 3.09 grams for the former and ± 2.85 grams for the latter. Thus, statistically, the greater growth increment is significant for the injected female rats and doubtful for the injected male rats.

A comparison of the mean growth increments of the animals in the first and second series (table 3) shows that the mean growth increments of the second series was greater than the corresponding increments of the first series. On the other hand, the weight differences between the experimentals and the controls were not as pronounced as in the first series. The injected male rats of the second series had a mean growth gain of 121.6 ± 2.78 grams and the injected female rats had a mean growth gain of 102.0 ± 4.42 grams. It may be observed from table 3 that this compares with a mean growth increment of 121.0 ± 2.82 grams in the male and 88.0 ± 3.04 grams in the female controls. Thus there is a greater growth difference of 0.6 gram in the injected males and 14.0 grams in the injected females. The probable errors are ± 3.53 grams for the former and ± 5.37 grams for

the latter. Again the increased growth gain for the injected female animals over their litter-mate controls appears to be significant.

In the third series (table 3) the extract-treated male rats had a mean growth increase of 50.2 ± 2.81 grams and the control male rats had a mean growth increase of 45.7 ± 1.79 grams. The mean growth gain was 41.8 ± 1.71 grams for the extract-treated female rats as compared to 34.0 ± 1.47 grams for the controls. Thus the mean growth differences were 4.5 grams for the males and 7.8 grams for the females. The probable errors are ± 3.33 grams for male animals and ± 2.26 grams for the female animals.

The fourth series of rats (table 3) received a daily injection of two cubic centimeters of cortico-adrenal extract, and this increased dosage might be expected to cause some variation from the results in the first three series. This factor, evidently, is of some significance, since the injected male rats had a mean growth increment of 70.2 ± 2.34 grams as compared to 63.1 ± 2.41 grams for the uninjected, and the injected females had a mean weight increment of 61.4 ± 1.51 grams as compared to 46.4 ± 1.73 grams for the uninjected. There was, therefore, a difference of 7.1 grams in favor of the injected males and 15.0 grams in favor of the injected females. The probable errors are ± 3.92 grams for the former and ± 2.29 grams for the latter difference.

It is to be observed that the extract-treated female rats in the four series gained in body weight faster than the males. Statistically, the values for the increased growth shown by the extract-treated animals are significant for females and doubtful for the males. On account of the apparent variation in the potency of the different batches of extract used, no quantitative correlation between increase in dosage and increase in percentage growth was possible. Likewise, the initial weights of the rats and the length of the experiment were not important factors in the difference in the mean body growth.

3. *Adrenalectomy and cortico-adrenal extract treatment. The estrous cycle of adrenalectomized rats.* Twenty-nine female rats were bilaterally adrenalectomized under ether anesthesia. These animals were selected from healthy stock which had shown regular ovarian cycles for at least two weeks previously. Daily vaginal smears were continued until the animals succumbed. No animal showed a positive estrual change more than one day after adrenalectomy and whenever this did occur, it conformed with the regular rhythm for that animal. All animals suffered gradual weight losses and died within the usual time. Conversely, twenty adrenalectomized rats treated adequately with cortico-adrenal extract maintained normal estrous cycles. This has been confirmed by a number of investigators (Corey and Britton, 1934; Martin and Kroc, 1933; Firor and Grollman, 1933).

In order to test the theory of Kroc and Martin (1934) that the return of estrus is secondary to the return to normal body weight, eight adrenalectomized female rats were allowed to lose weight for several days. No cortico-adrenal extract was administered until the animals appeared weak and had lost considerable weight. Daily vaginal smears were made and the animals were weighed each day. A positive estrous smear occurred from three to seven days after commencement of the extract treatment. In only one case did the body weight reach normal before estrus occurred. Two rats gained one gram only above the low figure at the beginning of extract treatment. Others revealed no marked change in weight; they were, indeed, far from reaching their normal weights. Disregarding the one exception, the weight regained before the onset of estrus was only 17 per cent, i.e., of the whole weight lost in the experiment. It is important to notice, however, that the body weight of every animal had increased. Although only a small series of animals was used, the indications are that cortico-adrenal extract had a direct effect on the restoration of the estrous cycle.

The ovaries and uteri of adrenalectomized rats. Twenty-eight young mature female rats were adrenalectomized and then were watched closely until they showed signs of extreme weakness. Some of them were prostrate and all were very weak before they were sacrificed. The average survival period was twelve days. The ovaries and uteri were weighed, fixed in formalin, and cleared in xylol.

A comparison of histological sections of ovaries of adrenalectomized rats with similar sections from the extract-treated adrenalectomized rats shows a marked difference. In the ovaries of animals very weak from adrenal insufficiency one finds no mature follicles, large and persistent corpora lutea, and very few small follicles; while in the ovaries of extract-treated animals there are some large follicles and many small follicles. Although corpora lutea may be present in the ovaries of extract-treated animals they do not occupy the greater proportion of the ovary. It would appear that degeneration of many follicles has taken place in the ovaries of adrenalectomized rats, and certainly no growth has occurred in the small follicles. The ovaries of the extract-treated adrenalectomized animals are normal. Repair and growth of follicles have occurred with the return of the normal estrous cycle.

Histological examination of the uteri of adrenalectomized rats showed a typical diestrous type of epithelium. In nearly every case the epithelium was low, there were few active uterine glands, and the normal folding within the lumen of the uterus was much reduced. It may be stated that in general the uteri gave the appearance of those from immature rats, rather than from animals of an adult age group.

In contrast to the uteri of rats suffering from adrenal insufficiency, the

uteri of the extract-treated adrenalectomized animals appeared normal. Histological sections showed medium or high epithelium cells, secretory glands, and normal folding of the uterine wall.

The testes of adrenalectomized rats. Fifteen young male rats, averaging one hundred and sixty-two grams, were adrenalectomized. Care was taken to observe these animals in the last stages of adrenal insufficiency. All were killed when they became very weak or were prostrate. The testes were weighed, fixed in formalin and cleared in xylol. Histological sections were made of one testis of each animal. These showed much disorganization and degeneration. Within the tubules the cells were loose, scattered, and disorganized and appeared in the lumen in some instances. Much more interstitial space occurred than in the normal testes. These pathological changes confirm the observations of other investigators (Freed, Brownfield, and Evans, 1931; Firor and Grollman, 1933).

The testes of twenty-five extract-treated adrenalectomized animals were improved. The tubules in histological sections showed less disorganization, and degeneration as a whole was less marked. A number of slides from the extract-treated animals showed little or no difference from normal material. These cases include the animals which had regained all or nearly all of their original body weight. It was found, therefore, that those animals which had improved most in body weight also had improved most as regards testicular structure. Although gain in body weight and testicular improvement were concurrent, the writer is of the opinion that this fact does not warrant the assumption that a return to normal body weight is essential to repair of the testes. Cortico-adrenal factors may or may not be involved.

4. *Estrogenic properties of cortico-adrenal extracts.* Adult female rats were selected at random from the cages and daily vaginal smears were taken for a period of two weeks. Only those rats showing regular estrual rhythms were selected for ovariectomy. Following the operation, vaginal smears were continued for a period of two weeks. Animals which showed no estrous stages later than two days after ovariectomy were selected for treatment. All injections were made subcutaneously, and with the exception of the first group which received only a small dose, the total amount of extract was divided into three even doses given at 9 a.m., 1 p.m. and 5 p.m. on one day. Vaginal smears were made 24, 48, 52, 56 and 72 hours after the first injection and then daily until the next test period. Only those smears showing cornified cells, or a mixture of cornified and nucleated cells, were considered as indicative of a positive reaction. The typical estrual stage, stage 4 of Long and Evans, containing round, cornified and nucleated cells, was not considered positive. Since no animal showed any estrual smear more than three days following the first injection of cortico-adrenal extract, ten days were considered a sufficient time interval between tests.

In order to determine whether increasing the dose would have any effect, several series of experiments were performed. In the first instance four cubic centimeters of cortico-adrenal extract were injected subcutaneously into each of twelve animals. In the second series twenty-six rats were treated with ten cubic centimeters each. For the third series the dosage was increased to twenty cubic centimeters. Finally, eight rats were given twenty-five cubic centimeters of cortico-adrenal extract each; and six were given twenty-five cubic centimeters of 1:1,000,000 solution of adrenalin each.

In the first series only two, or 16.7 per cent of the total, showed a positive estrual cycle. Stage 4 was encountered in one animal. Seven of the twenty-six rats in the second series, or 26 per cent, responded, and seven others had smears resembling stage 4. The result with the third series of twenty-six animals which received twenty cubic centimeters of cortico-adrenal extract was somewhat indicative. Ten, or 38.5 per cent, had positive vaginal smears. In the fourth series eight rats received twenty-five cubic centimeters of cortico-adrenal extract and only two responded. No response resulted from those receiving a control dosage of 1:1,000,000 adrenalin solution.

It is to be observed that a total of twenty-two positive responses were elicited out of seventy-two animals, that is, about thirty per cent of the cases. The use of large amounts of extract did not influence materially the percentage response. Based on the principle of Curtis and Doisy (1931), the results are negative.

Discussion. The stimulating effect of cortico-adrenal extract on the sex organs of immature female rats is in accord with other lines of evidence. It seems likely that there is a hormone secreted by the adrenal cortex which is concerned with sexual equilibrium. The inability to obtain positive results from the injection of this hormone into male rats is, possibly, due to the effect of impurities in the extract. Such impurities are found also in extracts of other tissues (Moore and Price, 1932; Robson and Taylor, 1933).

Although increased growth is evident in young rats treated with cortico-adrenal extract, it does not seem possible, with the cortico-adrenal extract which we have used up to this time, to develop giant rats or to bring about such profound growth as that which has been produced experimentally with extracts from the anterior lobe of the pituitary (Evans and Long, 1921). Corey and Britton (1931) produced hypertrophy of the pituitary gland in young rats with cortico-adrenal extract and assumed that the adrenal cortex acted by stimulating the pituitary to greater secretion. The growth gain is further indication that the hormone of the adrenal cortex initially stimulates the pituitary gland to increased activity; possibly the resultant excess pituitary growth hormone caused the observed

augmentation of the body weight. The increased sensitiveness of the female as contrasted with the male is in accord with the effects of the growth hormone of the anterior pituitary (Evans and Simpson, 1931).

It should be emphasized that cortico-adrenal extract contains no nutritive substances that might influence growth. The common tests for the proteins, carbohydrates, and fats, when made on the extract, are negative, except in those cases where phenolic derivatives are able to give positive tests (Swingle and Pfiffner, 1932). Furthermore, the total solid content of a cubic centimeter of extract is usually less than a milligram.

Observations upon adrenalectomized rats definitely show that a close connection exists between the adrenal cortex and the gonads. Adrenalectomized female rats may live for two weeks or more after the operation; however, they show no positive vaginal smears following operation. During this time the rats eat and give little outward signs of bodily disturbance. If one considers the difficulty of removing all adrenal tissue in rats, the occasional estrual cycle noted by most observers may be readily explained.

The report of Kroc and Martin (1934) to the effect that the restoration of the estrual cycle in adrenalectomized female rats with cortico-adrenal extract is dependent upon the return of body weight to normal is not confirmed. The estrual cycle was restored in all except one case before the body weight reached the pre-operative level. In all instances in this laboratory we have been able to restore the estrual cycle by adequate treatment with cortico-adrenal extract. The estrous cycle usually does not return immediately, but after a lapse of a few days. Fundamental degenerations take place in the ovaries, and this may well explain the delay.

Treatment of adrenalectomized rats with cortico-adrenal extract restores all sex organs to an apparently normal state. Histological sections of tissues of extract-treated animals show normal ovaries and uteri in contrast to sections of untreated adrenalectomized animals, which show ovaries with no growing follicles and large and persistent corpora lutea. Testicular regeneration rapidly follows adequate extract therapy. In some instances the organization is not complete. This depends in some degree, apparently, on the return of the body weight toward the pre-operative level, although normal histological sections of testes are obtained from some animals which have not reached their original body weight. The writer believes that the cortico-adrenal hormone which is concerned with sexual equilibrium has some effect upon the reorganization of the testicular structure, and that it is not entirely due to the general restoration of the body mechanism to normal.

In the case of adrenalectomized rats, the restoration of the estrual cycle does not in itself point definitely to an estrogenic substance. The effect on the ovaries may have been either direct or indirect and not of a truly

estrogenic character. In order to determine the certain presence of estrogenic properties in any extract it is necessary to use castrated animals. The method of Curtis and Doisy (1931) requires a response in seventy-five per cent of the treated castrated female rats for a positive reaction, or in other words, the detection of the presence of an estrogenic substance. Using this method we are unable to establish the presence of a true estrogenic substance.

SUMMARY

Hypertrophy of the ovaries and uteri of young female rats is produced by cortico-adrenal extract. Exact measurements of follicular and uterine diameters indicate that cortico-adrenal extract contains a female sex-stimulating factor.

Early maturity in the testes of cortico-adrenal extract-treated rats does not occur; indeed, inhibition of testicular growth results.

Cortico-adrenal extract stimulates growth in immature albino rats. Female animals that are treated with extract show a greater increase in body weight than do their male litter-mates.

Complete adrenalectomy suppresses the estrual cycle of female rats. Adequate administration of cortico-adrenal extract restores the normal estrual cycle. The return of estrus is not necessarily preceded by recovery of the normal body weight.

Marked atrophy of the reproductive system follows adrenalectomy. Pathological changes occur in the ovaries and testes. The ovaries have persistent corpora lutea and few growing follicles, while considerable degeneration and disorganization are present in the testes. Treatment with cortico-adrenal extract brings about repair in both ovaries and testes.

No specific effect of cortico-adrenal extract on the estrual cycle of castrated adult female rats is demonstrated. Positive responses occur in a number of cases; however, according to the method of bio-assay employed, they are not frequent enough to demonstrate the certain presence of an estrogenic substance in the extracts of the adrenal cortex.

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A STUDY OF THE WHITE BLOOD CELL PICTURE IN SIX YOUNG MEN

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Since, in a recent review, Garrey and Bryan (1935) have dealt admirably and very fully with the literature concerning the many factors which may alter the white blood count in man, it is necessary for us to emphasize only those investigations which seem to have a particular bearing on the present study. Reference to such literature will be made largely in connection with the discussion. Our purpose, in the experiment reported here, was to determine whether there might be any rhythm or pattern in the numbers of white blood cells in a series of six normal, resting, young men upon whom we made blood counts at fifteen minute intervals for five or seven hours on each of two successive days.

METHODS. The supravital method, employing neutral red as described by Sabin, Cunningham, Kindwall and Doan (1925), was used throughout the experiments for making the differential counts. Every precaution was taken to have all the glassware scrupulously clean and to use only dye films which were uniform so that no irregularity of staining could be due to faulty slides or dye.

During the entire series of experiments the same trained observer did all the supravital differentials. Two trained assistants did the total white counts, the same individual doing all the determinations in any one day's experiment. The same white pipette and the same counting chamber (each United States Bureau of Standards Certified) were used for all counts. Shaking the blood sample with the diluent (Türk's solution) was done by hand with a slight rotary motion; the resulting suspension of cells was very uniform. Both sides of the chamber were counted and the average number of cells used for calculations. In two instances only were duplicate counts made on a sample of blood because of an obvious faulty suspension.

Blood was obtained from the finger tips of the subjects by puncture with a knife blade protruding from a cork such a distance as to give a clean-cut wound, deep enough to allow a free flow of mixed venous and capillary blood. The first drops were always removed and in most cases blood was taken thereafter first, for the total white counts; second, for two fixed

smears; and last for the supravital films. The fresh smears were counted in a warm box at body temperature. Only one hundred white cells were differentiated in each instance because it was impossible for one individual working, to do more at fifteen minute intervals throughout a period of five to seven hours.

All the subjects' fingers were utilized for obtaining blood and at no time was an old puncture reopened or a new one put in its immediate neighborhood. This was done in an effort to avoid any error which might be introduced by drawing blood from an injured tissue area.

The subjects for the series of experiments were six normal men, twenty to twenty-five years of age, all except one being free from any manifest infections. The exception showed a constantly high eosinophilic count and was subsequently found to have a mild hookworm infection. For two successive mornings before the major experiment, each subject was put at rest in bed and total white cell counts were made at fifteen minute intervals for a period of three to four hours to determine his "basal" leucocyte count (Garrey and Butler, 1929) and also to discover whether the range was similar each day.

Prior to beginning each long series of counts, the subject was put to bed in the laboratory and made as comfortable as possible. He was allowed to read but not to move about excessively nor to get up except at intervals (noted on graphs). After half an hour, the counting and differentiation were begun. During the progress of the experiment each subject was allowed as much water as he would normally drink and between one and one thirty o'clock, a light lunch consisting of a sandwich and a milk shake.

When supravital spreads were made every effort was taken to procure as even a distribution of the blood as possible and only those preparations were counted where it was felt certain that the cells were not damaged by excessive pressure of the coverslip. In counting from the smears, complete excursions were made across the preparation for the purpose of obtaining an average distribution of cells. Other smears made from each sample of blood were later prepared with Wright's stain and the cells counted as a check for the supravital differentials.

The criteria for differentiating the types of cells encountered were those used by Sabin and her co-workers (1925). However, we did not separate from the granulocytic group the "non-motile" cell because in the limited time between counts we did not feel that we could distinguish with accuracy a truly non-motile leucocyte from one which we suspected to be suffering from mechanical injury by the coverslip.

RESULTS AND DISCUSSION. The results of the experiment were tabulated and graphs constructed from the information gathered on each individual. To avoid unnecessary complications the curves for successive days on each subject were plotted for comparison on the same graph and the total num-

bers of white blood cells, granulocytes, lymphocytes and monocytes were registered to the nearest hundred of cells. Analyses of the percentage numbers of the different granulocytes and lymphocytes and of the monocytes and the ranges of variation of these cells for each subject were tabulated and compared.

Because of the great general similarity between the graphs and also between the cellular analyses for all six subjects, only one representative graph (graph V) will be reproduced and two abbreviated tables (2 and 3) presented for cell differentiation and range variation respectively. Statistical information of total white blood cell counts for each of the six subjects appears in table 1.

TABLE 1

Statistical data on total leucocyte counts

SUBJECTS		NUMBER OF COUNTS	MEAN	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
						per cent
I	E. W. B. (1)	28	6585	±67	±522	±7.8
	E. W. B. (2)	28	6725	±63	±497	±7.3
II	F. L. O. (1)	29	5508	±76	±610	±11.0
	F. L. O. (2)	23	5919	±121	±863	±14.5
III	R. Z. Q. (1)	28	6578	±57	±449	±6.8
	R. Z. Q. (2)	28	6410	±59	±489	±7.6
IV	E. M. R. (1)	26	6307	±71	±539	±8.5
	E. M. R. (2)	28	6346	±124	±970	±15.2
V	C. W. W. (1)	28	6525	±51	±385	±5.9
	C. W. W. (2)	28	6660	±69	±542	±8.1
VI	G. H. F. (1)	28	7414	±118	±922	±12.4
	G. H. F. (2)	29	6218	±90	±715	±11.3

It was seen from observing all the curves for total leucocyte counts that they oscillated from count to count, but rhythm at a constantly recurring interval as mentioned by Sabin and her associates (1925) could not be demonstrated. Their observation that it is the numbers of granulocytes which impress their trend on the nature of the total leucocyte curves was corroborated. Shaw (1927), Jones and his co-workers (1933) and Smith and McDowell (1929) all noted fluctuations in their curves without regularity of rhythm. The last authors noted what they called a "pattern" which they believed characteristic for each individual whether on successive days or months apart. While the oscillations observed in the total leuco-

cyte curves in our cases tended to obscure their similarity, smooth curves drawn through the scatter of counts for each day showed that the undulations and trends of these curves for successive days maintained, within limits, the same pattern for a given individual (see graph V).

With four exceptions, in twelve curves, the total white counts fell after the subjects had been at rest for one-half to one hour, within the basal range previously determined for each individual thus substantiating the observations of Garrey and Butler (1929). In five of the six subjects the general trend of the total leucocyte curves on the second days was higher during the morning hours than on the first days. We believe that this might be due to emotional causes arising from the tedium of remaining

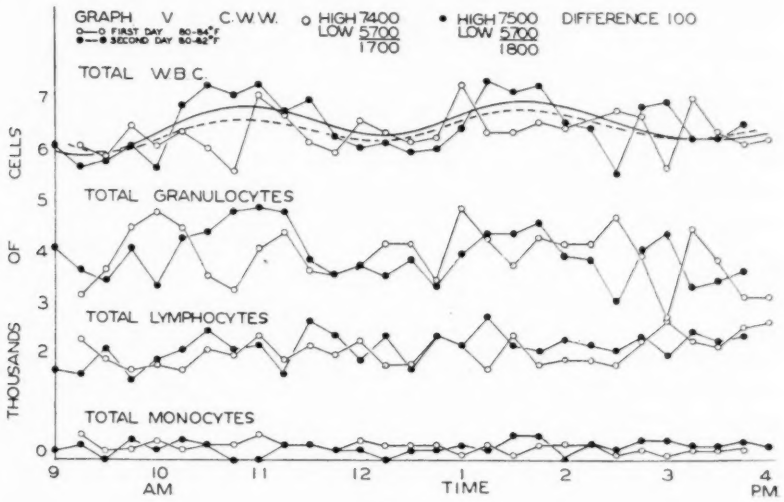

Fig. 1

quiet and the apprehension of having fingers stuck every fifteen minutes (Garrey and Butler, 1929; Zirm and Bauermeister, 1933; Bryan, Chastain and Garrey, 1932).

Alterations in leucocyte numbers due to subjects' assuming the erect posture after a period of rest were not marked in our series. There was a rise in seven instances, a fall in four; but the variations were not on the whole significantly great enough to mark them from any other oscillations in the curves. The greatest rise was 1400 cells, the greatest fall, 1200 cells while the least fall and least rise noted were 300 and 200 cells respectively.

For a given individual the difference in numbers of cells between highest and lowest counts of one day and of the same on the second day varied very little. For four rather phlegmatic individuals the range variation of

difference was 400, 200, 200 and 100 cells. In a fifth, highly volatile individual, the difference was 900 cells; while in the sixth a difference of 1800 cells was noted. This individual was, unknown to us at the time of doing the counts, enduring a "hangover" on the second day. Medlar (1931) has discussed the influence of alcohol on the white cell count.

A rise in the total number of leucocytes during the afternoon has been reported by different observers, yet in our cases there was no consistency in a steady rise lasting for several hours past noon. Out of twelve curves, three showed a marked upward trend; four, little change; and five, a downward trend. These findings are in accord with those of Ponder, Saslow and Schweizer (1931). In every instance, however, where a steady upward trend was not present, there was a demonstrable post-meridional peak between twelve and three o'clock. In several instances an ante-meridional rise was present (see graph V). Zirm and Bauermeister (1933) stated that there are leucocytic tides twice daily; one in the day which reaches a peak at 2 p.m. and a second which attains its height at 1 a.m. Similarly Shaw (1927) describes tides which are in flood during the afternoon and after midnight. The rather slight post meridional rises noted in many of our curves may represent the day-time phase of the diurnal tides just mentioned.

The minor waves of increased leucocyte numbers in the mornings and the short post-meridional waves probably should not be interpreted as digestional leucocytoses. From a review of the literature on this subject by Garrey and Bryan (1935), it seems clear that the routine ingestion of food in normal individuals has no effect upon the leucocyte counts. We feel that this is true in our cases as well, because the morning rises came long after breakfast and the post meridional rises had begun before our subjects had had lunch.

All that has been said above about the nature of the total leucocyte curves is applicable also to the curves of the total granulocytes. The lymphocyte curves, on the other hand, were in general mirror images of the granulocyte curves. In six of twelve curves, the lymphocytes tended to rise slightly but steadily throughout the duration of the experiment and in the other six curves to maintain an average level course. The monocyte curves in all cases maintained a practically unchanging level.

Following statistical treatment of our counts, table 1, the means of total leucocyte numbers for the subjects of our series are higher than those of Jones et al. (1933), but fall largely within the ranges of counts reported by Shaw (1927) and by Bryan and his associates (1935).

The average percentage numbers of different cell types and the percentage range of variation of cell types, derived from both supravital and fixed smear differentials for a typical example as well as the averages for all six cases will be found in tables 2 and 3. The correlation between counts by

the two methods is close, almost embarrassingly so. The percentages of granulocytes are higher than those reported by Sabin and her associates (1925) but similar to those of Jones et al. (1933) and Shaw (1927). The

TABLE 2

Average percentage table of the white blood cells from supravital and fixed smears

SUBJECTS			NEUTROPHILES	BASOPHILES	EOSINOPHILES	TOTAL GRANULOCYTES	SMALL LYMPHOCYTES	INTERMEDIATE LYMPHOCYTES	LARGE LYMPHOCYTES	TOTAL LYMPHOCYTES	MONOCYTES
V	C. W. W. (1)	S*	60.0	0.60	1.2	61.8	17.8	14.0	2.1	33.9	4.1
		F†	60.6	0.80	1.5	62.8	18.0	14.1	1.5	34.1	3.4
	C. W. W. (2)	S	59.9	0.40	1.5	61.8	18.7	14.0	1.4	34.1	4.0
		F	60.2	0.50	1.8	62.5	17.7	13.8	1.2	32.7	4.5
I to VI	First day	S	60.1	0.12	1.3	62.8	18.0	13.0	2.2	33.2	3.8
		F	60.5	0.80	1.6	63.6	16.6	12.9	2.7	32.3	4.0
Av.	Second day	S	61.5	0.18	1.4	63.8	17.4	13.0	2.8	32.9	3.1
		F	60.9	0.32	1.8	63.8	16.1	11.8	2.6	32.3	3.6

* Supravital smears.
† Fixed smears.

TABLE 3

Range of variation of different white blood cells in percentage in supravital and fixed preparations

SUBJECTS			NEUTROPHILIC LEUCOCYTES	BASOPHILIC LEUCOCYTES	EOSINOPHILIC LEUCOCYTES	TOTAL GRANULOCYTES	SMALL LYMPHOCYTES	INTERMEDIATE LYMPHOCYTES	LARGE LYMPHOCYTES	TOTAL LYMPHOCYTES	MONOCYTES
V	C. W. W. (1)	S*	47–70	0–2	0–3	48–70	13–29	9–23	0–9	27–50	2–8
		F†	49–70	0–2	0–3	50–70	10–28	6–20	0–10	26–45	0–7
	C. W. W. (2)	S	53–68	0–2	0–3	55–68	14–22	17–20	0–6	26–41	1–7
		F	50–69	0–2	0–4	53–70	10–23	8–23	0–6	22–42	0–8
Average I to VI		S	54–67	0–1.5	0–3.7	56–71	10–25	8–19	0–9	26–40	0.5–7
		F	52–68	0–1.9	0.1–3.9	56–71	8–24	7–21	0–9	25–40	0.6–7

* Supravital smears.
† Fixed smears.

average percentage cellular composition of the blood in our six subjects was almost the same for each individual on successive days although the ranges of variation were not quite so similar.

SUMMARY AND CONCLUSIONS

We have presented a study of the blood cell counts of six resting young men, taken every fifteen minutes for five to seven hours on two successive days. While marked oscillations appeared in the curves as the result of fluctuations in total numbers of leucocytes, we were not able to establish proof of rhythmicity occurring at regular intervals of short duration, either for the total numbers of leucocytes or for total numbers of any one cell type of the blood.

The character of the total leucocyte curve as has been previously shown is determined by the numbers of granulocytes.

If smooth curves are drawn through the scatter of leucocyte counts the pattern of curve is similar for a given individual on successive days as is also the average percentage cellular composition of the blood.

Although sustained afternoon rises in the total leucocytes were an inconstant feature, post-meridional rises of short duration were commonly present and may represent the daytime phase of diurnal tides noted by other observers.

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BLOOD AND MUSCLE LACTIC ACID IN THE STEADY STATE

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In the paper of Hill, Long and Lupton (1924) on muscular exercise, lactic acid and oxygen, the following statement is made: "We will assume, moreover, that when the steady state of exercise has been fully attained, the lactate ion concentration is the same in muscle as in blood plasma . . . ." A similar statement is made by Margaria, Edwards, and Dill (1933): "Thus, except when changes in lactic acid production are so rapid that an equilibrium between the concentrations of lactic acid in the blood and tissues has not been reached, we are justified in assuming the concentration of lactic acid in the blood to be proportional to the total amount of lactic acid in the body. . . . " In this work it was assumed that in the steady state, the lactic acid present was uniformly distributed throughout the body water.

This assumption is of the greatest importance with regard to the physiology of exercise in man and in relation to the chemistry of muscular contraction. For example, the conclusions of Hill, Long, and Lupton on the rôle of lactic acid in contraction and its fate in recovery depend on the correctness of this assumption. And this assumption, among others, is one on which Margaria, Edwards, and Dill have postulated an "alactacid" mechanism for performing muscular work anaerobically. The possibility of calculating an "oxidative quotient" for lactic acid in recovery, as both these groups have done, depends on the validity of the assumption.

For these reasons, and because some other experiments had indicated rather slow passage of lactic acid from muscles to blood, conditions were sought which would permit the assumption to be put to the test on the laboratory animal, since the direct experiment cannot be made on man. For this, it is necessary to have a muscle in the steady state of activity, with the arterial blood reaching it having a constant lactic acid concentration throughout the entire period of the experiment. If the plasma of the venous blood issuing from the working muscle under these conditions contains approximately the same concentration of lactic acid as the muscle itself does, the assumption is justified. On the other hand, if the venous plasma contains lactic acid in significantly lower concentration than the

muscle does, the assumption fails and conclusions based on it must be subjected to critical re-examination. Plasma is used rather than whole blood, as it is known that corpuscles contain a lower concentration of lactic acid than the plasma in which they are suspended.

The gastrocnemius muscle of the cat, subjected to one or two isometric twitches a second, reaches the steady state, so far as twitch tension and lactic acid concentration are concerned, within two minutes of the onset of stimulation, and remains thus for at least ten minutes (Sacks, Sacks and Shaw, 1937). When only this muscle is undergoing stimulation, the requirement of constancy of arterial blood lactic acid is met, since the mass of muscle producing lactic acid is quite small relative to the mass of the animal and the capacity of the mechanisms for removing lactic acid from the blood. Experiments were performed on cats under pentobarbital anesthesia. Both gastrocnemius muscles were dissected out, leaving origins and blood and nerve supply intact. In one leg all branches of the popliteal vein except those emerging from the gastrocnemius muscle were tied off, and the tibial and peroneal nerves were cut just distal to this muscle, which was then prepared for indirect stimulation. Stimulation was begun, and after three minutes a blood sample was taken from the carotid artery. Immediately after, the venous blood issuing from the contracting muscle was collected. When a sufficient amount had been obtained, the muscle was frozen. Stimulation was stopped only after the freezing mixture had been poured over the muscle. The resting muscle was then frozen. In the experiments in which venous blood was collected from the resting leg, it was taken from the popliteal vein. Collection was started after the first minute of stimulation, and three to four minutes were required to obtain an amount sufficient for analysis. Sodium fluoride and potassium oxalate were used to inhibit glycolysis and prevent coagulation. Lactic acid determinations on trichloracetic acid filtrates of muscle and plasma were made by the method of Friedemann, Cotonio and Shaffer (1927).

The results of these experiments, which are shown in table 1, were, without exception, at variance with the assumption of uniform distribution of lactic acid in the steady state. In no case was the lactic acid concentration in the plasma so much as one-fourth of that in the muscle from which the blood had just emerged, and with very high lactic acid concentrations in the muscles, the disproportion was even greater.

Since Eggleton and Evans (1930) have reported very marked rises in (arterial) plasma lactic acid within a short period after repeated tetanic stimulations, it was decided to investigate the situation with respect to venous plasma during the early part of the recovery period. For this, the same procedure was used, except that as soon as sufficient blood had been obtained from the muscle, stimulation was stopped and the venous blood

of the first two minutes of recovery collected separately; the muscle was then frozen, within five seconds after stopping the collection of blood. Tetanic stimulation was used in a few experiments in this group, instead of repeated single shocks; in these cases the collection of blood was begun 30 seconds after commencing stimulation. Here, also, the blood of the first two minutes of recovery was collected separately and the muscle then frozen.

These experiments, the results of which are shown in table 2, show only a somewhat smaller disproportion between the lactic acid contents of plasma and muscle than was found in the first group. The lactic acid content of the venous plasma early in recovery is somewhat higher than

TABLE 1

Lactic acid concentrations in plasma and muscle during steady state of activity of a single muscle (cat gastrocnemius)

Values are in milligrams per cent of lactic acid

EXPERIMENT NUMBER	ARTERIAL PLASMA	RESTING VENOUS PLASMA	RESTING MUSCLE	STIMULATED VENOUS PLASMA	STIMULATED MUSCLE
a. Muscles stimulated twice per second					
1	9	7	11	27	322
2	9	8	10	33	284
3	13	15	17	59	324
4	9	11	13	24	224
b. Muscles stimulated once per second					
5	6		10	15	95
6	10		9	22	109
7	6		7	22*	104
8	8		9	29	163

* Whole blood.

during the steady state, as might be expected from the slower circulation, but it still fails to approach that of the muscles.

Although the experiments on cats gave such clear-cut results, it was considered desirable to repeat them on another species. The attempt was made to duplicate exactly the experimental procedure, using rabbits instead of cats. However, this was found not to be feasible. By stimulating the entire leg musculature through the intact sciatic nerve, and collecting blood from the femoral vein, an approximation to the conditions reached in the experiments on the cat was possible. In this case, however, the condition of constant arterial blood lactic acid disappeared, for, with the larger muscle mass undergoing stimulation, the total amount of lactic acid added to the blood apparently became greater than could be removed

by the combined action of the non-working muscles (Barr and Himwich, 1923) and the liver (Himwich, Koskoff and Nahum, 1928). The results of these experiments, shown in table 3, are confirmatory of those obtained on the cat, although the disproportion in lactic acid concentrations between plasma and muscle is not nearly so great in the rabbit as in the cat.

TABLE 2

Lactic acid concentrations in plasma and muscle in first two minutes of recovery from steady state activity in a single muscle (cat gastrocnemius)

Values are in milligrams per cent of lactic acid

EXPERIMENT NUMBER	ARTERIAL PLASMA	RESTING MUSCLE	STIMULATION VENOUS PLASMA	RECOVERY VENOUS PLASMA	STIMULATED MUSCLE
a. Twitches at rate of 2 per second					
9	9	18	29	37	291
10	10	13	53	69*	265
11	10	18	24	26†	196
12	12	8	30	29	146
b. Tetanus					
13	12	21	47	58	126
14	7	22	50	52	133
15	14	8	27	37	171

* Whole blood.

† One minute of recovery.

TABLE 3

Plasma and muscle lactic acid concentrations in steady state of activity of group of muscles (leg of rabbit)

Values are in milligrams per cent of lactic acid

Stimulation of sciatic nerve at rate of 2 per second

EXPERIMENT NUMBER	ARTERIAL PLASMA	RESTING MUSCLE*	STIMULATED VENOUS PLASMA	STIMULATED MUSCLES		
				Gastrocnemius	Flexor digitorum longus	Tibialis anterior
16	54	25	83	118	189	213
17	35	18	81	148	144	93
18	55	21	90	97	132	177
19	35	35	122	144		274

* Tibialis anterior.

However, these experiments do add another point which is worthy of note: two different muscles in the same extremity, stimulated simultaneously through the nerve, are found to contain markedly different concentrations of lactic acid. Thus, not only does the blood fail to indicate the lactic acid concentration present in the muscles, but also one

muscle cannot necessarily be used to indicate the lactic acid content in another muscle subjected to the same intensity and duration of stimulation. This factor probably accounts for some of the irregularities in the data of Eggleton and Evans.

It now becomes necessary to re-examine the theoretical conclusions which have been drawn from data based on the assumption of uniform lactic acid distribution. First, there is the "oxidative quotient" for lactic acid. This has been calculated from the extra oxygen consumed and the decrease in lactic acid content of blood or plasma in recovery from exercise. Obviously, if the concentration in the blood at the end of the exercise is only a fraction of that in the muscles, there is no way of telling how much lactic acid was removed from the body for each liter of extra oxygen consumed.

Another concept requiring evaluation is the "alactacid" oxygen debt postulated by Margaria, Edwards and Dill, and further studied by Margaria and Edwards (1934). The findings on which these workers base their concept of an "alactacid" mechanism for performing work anaerobically are these: 1, in a trained athlete running on a treadmill at constant rate for 10 minutes, no appreciable amount of lactic acid appeared in blood taken from an arm vein, at levels up to 10 times the basal oxygen consumption, even though an oxygen debt was incurred; and 2, in successive periods of running at high rate in an untrained individual, with rest periods of 5 minutes, the increment in arm vein blood lactic acid was less after the subsequent work periods than after the first. They ascribe the oxygen debt in these cases to some process yielding energy anaerobically and not involving the formation of lactic acid. However, in the light of the present findings, such an assumption is no longer necessary. In the first type of experiment, it would be expected that in an athlete in training, as was the case with their subject, the cardiac and respiratory adjustments might be made rapidly. Hence little lactic acid would be formed in the muscles before the work began to be performed by means of energy derived from oxidative reactions. With a low diffusion gradient present, the liver and the non-working muscles might easily remove the small increments of lactic acid added to the blood by the working muscles, and no appreciable rise in blood lactic acid would be expected. In the second instance, the assumptions are made that the capacity of the muscles to form lactic acid is exhausted in the first work period, and that the work in the subsequent periods is practically completely anaerobic, even though the oxygen consumption at the end of the rest period is three times the resting level. These assumptions are not acceptable without reservations, nor does the blood from an arm vein five minutes after the subject has run on a treadmill necessarily contain the same lactic acid concentration that any of the muscles of the legs do. It is therefore unnecessary to postulate any "alactacid" mechanism.

It may also be pointed out that the low diffusion rate of lactic acid from muscles to blood is of great advantage to the organism. If equilibrium were to be as readily reached with respect to lactic acid diffusion as it is with respect to carbon dioxide and oxygen, then a relatively short period of vigorous exercise in an untrained individual might reduce the alkali reserve of the blood to the vanishing point. Thus the lack of free diffusion of lactic acid is seen to be another factor of safety to enable the body to withstand temporarily adverse conditions without too great a departure from the conditions of homeostasis.

SUMMARY AND CONCLUSIONS

1. During the steady state of muscular activity, the plasma of the venous blood issuing from the muscles contains a significantly lower concentration of lactic acid than does the muscle itself.

2. In the early part of the recovery period, the lactic acid content of the plasma remains much lower than that of the muscles.

3. These findings show that it is not possible to calculate an "oxidative quotient" for lactic acid in man on the basis of changes in blood lactic acid.

4. These findings also make it unnecessary to postulate an "alactacid" mechanism for performing work anaerobically.

5. The lack of free diffusion of lactic acid is shown to favor the maintenance of homeostasis in vigorous exercise.

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THE EFFECT OF VARIATIONS IN TOTAL CALCIUM CONCENTRATION UPON THE COAGULATION TIME OF BLOOD

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Numerous investigators have studied the relationship of calcium to blood coagulation (1). While calcium is recognized as essential for normal coagulation, there is no evidence to be found in the literature of the exact amounts of calcium required. Such studies as have been made deal for the most part with blood treated with oxalate or other anticoagulants. Since the mechanism by which these anticoagulants, particularly oxalate, produce their effect is not entirely clear, any calculations as to the quantity of available calcium in blood so treated must be somewhat uncertain, and the deductions from such studies are consequently inconclusive.

Clinical and animal investigations for the most part have offered no grounds for believing low blood calcium to be a cause of increased coagulation time. Ravdin and Morrison (2) studied a series of dogs with serum calcium values of 4 to 8 mgm. per 100 cc. and found no evidence of prolongation of clotting time. Ravdin, Riegel and Morrison report one patient with parathyroid tetany and serum calcium of 4.6 mgm. per cent but normal coagulation time. Kottman and Lidsky (3) reported decrease in coagulation time in parathyroidectomized dogs but retarded coagulation in a patient with parathyroid tetany. One case of hemorrhagic disease apparently due to hypocalcemia in a nutritional anemia was reported by Snyder (4).

The relation between calcium and the hemorrhagic tendency of certain cases of obstructive jaundice has attracted attention in recent years. A review of the subject has been given by Carr and Foote (5). There seems to be no adequate evidence that a deficiency of calcium is responsible for the bleeding.

While the investigations reported offer no evidence of a definite relationship between calcium concentration and coagulation time within the range of calcium concentrations observed physiologically, it seemed worth while to make a more systematic study of the subject to determine the amount of calcium necessary for normal coagulation and the effects of variation in total calcium concentration upon coagulation time.

PROCEDURE. To avoid confusion resulting from the addition of anticoagulants these experiments made use of untreated whole blood. Since it is generally agreed that the rôle of calcium in coagulation is in the conversion of prothrombin into thrombin, it is important that no platelet disintegration occur to initiate thrombin formation before the desired experimental conditions are produced. The blood was therefore drawn from normal adults into chilled oiled syringes and transferred immediately to paraffined tubes standing in ice. Measurements were made and blood transferred to the diluting solutions with chilled paraffined pipettes. Since paraffin interferes with any accurate calibration of pipettes, the measurements were in drops from capillary pipettes delivering an approximately constant size of drop.

It was determined in preliminary experiments that in a dilution of 1:10 with normal saline blood will not coagulate, but if calcium is added, a firm clot is formed. This proportion of blood to diluting solution was consequently adopted for the experiments, four drops of blood being added to thirty-six drops of a calcium-saline mixture. The calcium solutions were prepared by dissolving $CaCl_2$ in normal saline, and both the calcium and saline solutions were adjusted to a pH of approximately 7.6 by the addition of a trace of $NaHCO_3$, in order that coagulation time might not be influenced by differences in hydrogen ion concentration.

In a series of paraffined tubes (1 x 10 cm.) were placed calcium and saline solutions in proportions to give the desired calcium concentration, the total amount equalling thirty-six drops. As soon as possible, usually within ten to fifteen minutes, after the blood was drawn it was transferred to the calcium mixtures, four drops to each tube. The tubes were shaken to mix thoroughly and allowed to stand at room temperature about fifteen minutes. After this each tube was tipped once every minute and the time of coagulation noted. The time of appearance of a firm clot was taken as the coagulation time.

RESULTS. Individual experiments practically always showed some irregularities in coagulation time and did not give smooth curves. This is probably due to the difficulty of keeping all factors except calcium absolutely constant. The slightest difference in handling the blood can affect the rate of platelet disintegration. If the blood touches the side of the paraffined tube when being added to the diluting solution or if there is an irregularity in the paraffine lining tube or pipette a decreased coagulation time may result. By taking a sufficiently large series of experiments, however, the average values obtained should give an accurate picture of the effect of calcium concentration on the coagulation time. Table 1 gives the results of two such series of experiments.

It is evident from these figures that variations between 5 and 20 mgm. per cent in the concentration of calcium have no significant effect upon the

coagulation time of blood. Below 5 mgm. per cent coagulation begins to be retarded but it is not until the calcium is reduced to 2.5 mgm. per cent, or below, that differences appear which might be of importance in coagulation time.

TABLE 1

Effect of calcium concentration on coagulation time of blood

CALCIUM CONCENTRATION	COAGULATION TIME. AVERAGE OF 32 DETERMINATIONS. DILUTION 1:10	COAGULATION TIME. AVERAGE OF 10 DETERMINATIONS. DILUTION 1:13
mgm. per cent	*minutes*	*minutes*
36.5	31	
30.5	27	
20.5	24	24
18.5	28	
16.5	29	
12.5	30	
10.5	30	22
8.5	28	
7.5	28	
6.5	28	
5.5	29	24
4.5	28	
3.5	30	
2.5	32	25
1.5	62	60
1.0	>65	
0.5	>120	

TABLE 2

Comparison of coagulation time of natural plasma diluted with normal saline with that of similar dilutions with 10 mgm. per cent calcium in saline

A. Tubes in which plasma is diluted with normal saline. B. Tubes in which plasma is diluted with 10 mgm. per cent calcium in saline. Average of six determinations.

PLASMA	DILUENT	ESTIMATED CALCIUM CONCENTRATION IN A	PROLONGATION OF COAGULATION TIME OF A OVER B
cc.	*cc.*	*mgm. per cent*	*minutes*
0.8	1.2	4	+3
0.6	1.4	3	+4
0.4	1.6	2	+9
0.2	1.8	1	+109
0.1	1.9	0.5	00

The clots were usually not firm in tubes containing only 1 mgm. per cent calcium but in the higher concentrations firm clots were nearly always formed.

These experiments, of course, consider only the total calcium present without regard to whether it is in a diffusible or non-diffusible form. If the non-diffusible calcium of the blood is in combination with plasma proteins, an increase in the concentration of protein in the mixture should increase the proportion of non-diffusible calcium. In order to test whether the influence of total calcium upon coagulation time would be altered by increasing the proportion of plasma protein present, a series of experiments was run using twelve drops of blood diluted to forty instead of four drops as in the previous experiments. A few determinations were also made in which the desired calcium concentrations were obtained simply by diluting natural plasma with the necessary amount of normal salt solution, and comparing the coagulation time with that of plasma similarly diluted with 10 mgm. per cent calcium in saline. Here the proportions of plasma proteins are different for each concentration of calcium (see table 2).

In neither of these two series of experiments do the curves differ materially from those obtained with blood diluted 1:10. They offer no evidence of a difference in the influence of calcium on coagulation time in the presence of varying proportions of plasma protein.

The experiments presented here demonstrate that, at least in vitro, variations in total calcium concentration between 5 and 20 mgm. per cent have no significant effect upon coagulation time. Such changes in the proportion of diffusible and non-diffusible calcium as may be produced by variations in the amount of plasma protein present are apparently without influence on the relation of total calcium to coagulation time.

The evidence of these experiments supports the results of the investigations previously discussed, which tend to show that prolonged coagulation as observed in hemorrhagic conditions cannot be the result of a low blood calcium alone.

SUMMARY AND CONCLUSIONS

A series of experiments has been presented in which the influence of variations in total calcium concentration upon the coagulation time of blood was determined.

Coagulation time is practically constant with calcium concentrations between 5 and 20 mgm. per cent. Marked prolongation of coagulation time appears first with calcium reduced to 2.5 mgm. per cent or less.

Variations in amount of plasma protein present, which might alter the proportions of diffusible and non-diffusible calcium, do not affect the relationship between total calcium and coagulation time.

In view of these results, prolonged coagulation time as observed in hemorrhagic conditions cannot be interpreted as ever being the result of low blood calcium alone.

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EFFECT OF MOTION ON THE ELECTRICAL CONDUCTIVITY OF THE BLOOD

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Measurements of the electrical conductivity of blood have been made, as far as we are aware, only in vitro on resting blood (1, 2, 3, 4, 5). We found that the electrical conductivity is altered when the blood is put in motion. This report deals with studies made in an attempt to analyze this interesting phenomenon.

PROCEDURE. The electrical conductivity measurements were made with an alternating current bridge circuit (6). A 1000 cycle General Radio oscillator was employed as a power source, and the point of balance in the bridge was determined by adjusting to obtain the minimum sound in the ear phones. The sharpness of this point was greatly increased by inserting a "blood impedance" in series with the variable decade resistance box used to measure conductivity. This was done to balance the capacity on both sides of the bridge.[2] This "blood impedance" consisted of a test tube of the same blood as used in the experiments, into which two copper wires were inserted close to one another; the length of wire in the blood was adjusted until the hum in the ear phones at the minimum adjustment of the bridge almost completely disappeared. It was found that this point was obtained when the area of this wire in contact with the blood was the same as the electrode area in contact with the blood in the flow tube.

Fresh defibrinated beef blood (obtained through the courtesy of Dr. D. Klein of the Wilson Laboratories) was used. This was filtered several times through fine muslin to remove clots and was allowed to come to room temperature. The measurements were made for the most part with glass tubes with a pair of circular platinized platinum electrodes one

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[2] This blood compensator had to be readjusted between experiments from time to time which introduced small variations of the absolute value of the resistance on the corresponding side of the bridge. This was satisfactory for our purposes since we were interested in resistance variations in single experiments rather than in absolute values.

square centimeter in area, one at each end. Blood entered the tube from a glass reservoir. To avoid settling, the blood in the reservoir was agitated from time to time. The rate of blood flow was altered by means of a screw clamp on the rubber inflow tube, and the outflow was measured in cc./sec. with a graduate and stop watch. This was converted into linear velocity of flow in cm./sec. The electrical conductivity measurement during flow was compared with the stabilized reading on the blood at rest before and after the blood was put in motion. "Specific" resistance was calculated from these measured values and the dimensions of the tube. We paid attention only to alterations in ohmic resistance as this proved to be the component of the impedance primarily responsible for the observed effects (see below).

DISCUSSION OF RESULTS. *Whole blood.* Over 50 samples of beef blood and five samples of defibrinated dog's blood were tested roughly, and it was found that the electrical resistance decreased in all when the blood was put in rapid motion. In most samples of beef blood, at slow flows the resistance increased; this did not occur with the dog's blood. Eight samples of beef blood were examined more carefully at various velocities; three typical curves are shown in figure 1. The "specific" resistance of these blood samples varied at rest from 174 to 164.5 ohm-cm. (cf. table 1). The shape of most of the curves (such as the top and bottom curves of fig. 1) suggests that two opposing effects are involved, one being the tendency of the resistance to rise, which is predominant at slow flows and gradually overshadowed at faster flows by the effect of a decreasing electrical resistance with increasing rates of flow. In several curves this first effect was absent (viz., middle curve of fig. 1). The decrease in resistance is not linearly proportional to the velocity of flow, but the curve gradually approaches a horizontal line at fast flows. The increase of resistance at slow flows we have called the electrode effect (see below); the decrease of resistance at faster flows we have called, for short, the flow effect. It is noteworthy that the flow effect at a given rapid velocity is less in those samples having a large electrode effect than in those without the electrode effect (cf. table 1). In several samples, the flow effect was tested in tubes of different sizes, and, provided the same type of electrodes was used, the flow effect at different velocities was found to fit a single curve within the experimental error of the measurements (cf. top curve of fig. 1). The increased resistance at slow flows disappeared when the electrodes were removed from contact with the moving blood and placed in contact with stationary blood or saline in side arms communicating with the moving blood; the flow effect at fast flows in the same blood samples, however, remained unaltered in character. This increased resistance at slow flows also failed to appear when beef blood was passed through a dog's aorta obtained postmortem, with the elec-

trodes placed in contact with its outside wall. The decreased resistance at faster flows was present, however. (Change in calibre of the blood vessel was prevented by inserting in the vessel a tightly fitting glass tube having two small perforations.)

In view of these results, the increased electrical resistance at slow flows is dependent in some unknown fashion on an interaction between certain types of electrodes with some samples of moving blood. It is an electrode effect, depending on relative motion of blood past an electrode. It will not be discussed further.

The decreased electrical resistance is independent of the nature of the electrodes and of the size and shape of the measuring vessel. It is determined by the character of the blood and the linear velocity of blood motion. Experiments showed that the temperature coefficient of the

TABLE 1

SAMPLE OF WHOLE BEEF BLOOD	"SPECIFIC" RESISTANCE AT REST	"SPECIFIC" RESISTANCE OF FLOW AT A LINEAR VELOCITY OF 15 CM./SEC.	FLOW EFFECT	"ELECTRODE EFFECT"*
	ohm-cm.	*ohm-cm.*	*ohm-cm.*	*ohm-cm.*
1	171.0	162.0	9.0	1.0
2	167.5	159.0	8.5	0.0
3	170.0	162.0	8.0	0.0
4	173.5	166.0	7.5	2.0
5	174.0	167.5	6.5	3.0
6	174.0	169.0	5.0	3.0
7	172.5	168.0	4.5	3.0
8	164.5	161.5	3.0	3.0
Range.....	164.5	159.0	3.0	0.0
	174.0	169.0	9.0	3.0

*Maximum increase in "specific" resistance at slow flow.

blood resistance and the possible small temperature variations are not great enough to account for the observed effect. A rough estimate showed that the temperature coefficient of the "specific" resistance in one sample of blood, in the range between 18° and 39°C., was of the order of -1.5 ohm-cm. per each degree rise. Pressure changes (such as might occur when the blood in the measuring tube is suddenly connected and disconnected from the blood reservoir) produced no measurable change in electrical resistance.

The similarity of results obtained with galvanic and alternating current shows conclusively that the main factor involved must be an alteration of the ohmic resistance. The experiment dealing with this phase was as follows: A special measuring tube with side arms filled with saline was used. In these side arms silver–silver chloride electrodes were inserted.

The usual method of measuring the flow effect with alternating current was employed. The same circuit was also used for the galvanic current except that the current source consisted of two dry cells giving 3 volts, and the null point was determined by a moving-coil mirror galvanometer. The results are given in table 2. It will be seen that with both types of current the slow flow had no effect and fast flows caused the resistance to decrease the same amount within the experimental error (viz., 75 ohms with alternating current and 70 ohms with the galvanic current). The decreased resistance at rest with the alternating current is partly due to a decrease in the capacitative reactance of the erythrocytes in conducting alternating current; a decrease which becomes more marked as the frequency of the alternating current is augmented.

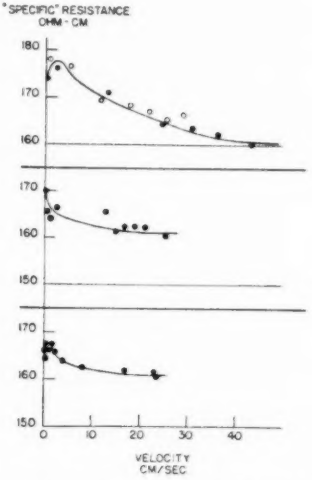

Fig. 1. "Specific" resistance of three samples of beef blood at various blood velocities. In top curve, solid and open circles represent, respectively, values obtained with a small and large sized measuring tube. Discussed in text.

Our results show that this flow effect is limited to blood; no other solution, suspension or emulsion which we used gave any measurable flow effect in the velocity range of our blood measurements. The following fluids were tested: *a*, normal Ringer's solution; *b*, 0.9 per cent saline; *c*, cows' milk; *d*, 50 per cent colloidal $FeSO_4$; *e*, 50 per cent colloidal Al $(C_2H_3O_2)_2OH$; *f*, 50 per cent petroleum emulsion; *g*, a suspension of uncoated glass particles (5–7μ in diameter) in 0.9 per cent NaCl (60 parts of glass particles to 40 of NaCl); *h*, a suspension of gelatine coated glass particles (same diameter as *g* above) in 0.9 per cent NaCl (proportion of glass particles to NaCl as in *g*); *i*, a 50 per cent suspension of chalk in 0.9 per cent NaCl.

The component of the blood responsible for the flow effect. Two different samples of blood were centrifuged to separate the erythrocytes and serum, and measurements were made on each fraction. It was found that no change in electrical resistance occurred when the serum was put in motion, its "specific" resistance remaining constant at an average of 74 ohm-cm. in the two samples. Changes in electrical resistance were obtained, however, when the erythrocyte fractions were put in motion. The effect was much greater than that obtained with the original blood from which the samples were taken, but the curves relating the flow

effect to the linear velocity were of the same type. The "specific" resistance at rest of the erythrocyte fractions was 233 and 310.5 ohm-cm. compared with 174 and 166 ohm-cm. respectively for the original blood from which they were obtained. The decrease of electrical resistance at 15 cm./sec. flow was greater than for the original blood samples, viz., 17.5 and 25.5 ohm-cm. compared with 6.5 and 4 ohm-cm. This flow effect disappeared when the erythrocytes were hemolyzed, the resistance of the hemolyzed portion being approximately the same as that of serum. Apparently the change in electrical resistance with the blood in motion is due to the presence of formed erythrocytes in their natural state.

Our results on the erythrocyte fraction do not support the assumption adopted by Stewart (1) and accepted by Fricke (2, 3) that the erythrocytes have a tremendously greater resistance than serum. The erythrocyte fraction, while not being entirely free of serum, was as nearly so as we could make it, and its resistance was more of the order of $3\frac{1}{2}$ to

TABLE 2

		RESISTANCE AT REST BEFORE FLOW MEASUREMENT	RESISTANCE WHILE FLOWING	RESISTANCE AT REST AFTER FLOW MEASUREMENT
		ohms	*ohms*	*ohms*
Fast flow	Alternating current	3720	3650	3730
	Galvanic current	4540	4480	4560
Slow flow	Alternating current	3710	3710	3710
	Galvanic current	4530	4540	4550

5 times that of serum at this frequency (1000 cycles per second). This is more in accord with the views expressed by Brooks (4).

The action of altering the concentration of erythrocytes on the flow effect. That the flow effect was due to the erythrocytes was shown also by the greater effect obtained when the concentration of the erythrocytes was varied from 5 to 100 per cent by volume. (The 100 per cent sample was as nearly free of serum as it is possible to obtain by high speed centrifuging for over an hour. It had some serum in it, but probably less than 1 per cent.) This was done on four samples of beef blood. In each the serum and erythrocytes were separated by high speed long period centrifuging. The two were then mixed in different proportions and the flow effect on each determined. The results are summarized in table 3 and the averages are plotted in figure 2. The upper curve in figure 2 represents the average values for the various concentrations when at rest; the middle curve gives the average values when the samples were put in motion at a velocity of 15 cm./sec.; and the bottom curve gives the average flow

TABLE 3

PER CENT SUSPENSION OF BEEF ERYTHROCYTES IN BEEF SERUM	SAMPLE NO.	"SPECIFIC" RESISTANCE AT REST	"SPECIFIC" RESISTANCE OF FLOW AT A LINEAR VELOCITY OF 15 CM./SEC.	FLOW EFFECT
		ohm-cm.	*ohm-cm.*	*ohm-cm.*
100 per cent erythrocytes suspension	1	233	215.5	−17.5
	2	—	—	—
	3	—	—	—
	4	310.5	284.0	−26.5
	Average	271.75	249.75	−22.0
80 per cent erythrocytes suspension	1	181.5	174.5	−7.0
	2	204.5	195.0	−9.5
	3	200.0	196.0	−4.0
	4	200.0	191.5	−8.5
	Average	196.5	189.25	−7.25
60 per cent erythrocytes suspension	1	140.0	137.0	−3.0
	2	147.5	142.5	−5.0
	3	152.0	150.5	−1.5
	4	142.0	139.0	−3.0
	Average	145.4	142.25	−3.15
40 per cent erythrocytes suspension	1	111.0	110.0	−1.0
	2	127.0	123.0	−4.0
	3	121.0	121.0	0.0
	4	105.5	104.5	−1.0
	Average	116.15	114.65	−1.5
20 per cent erythrocytes suspension	1	89.0	89.0	0.0
	2	99.0	98.0	−1.0
	3	96.0	97.0	+1.0
	4	82.0	81.5	−0.5
	Average	91.5	91.35	−0.15
5 per cent erythrocytes suspension	1	—	—	—
	2	82	82	0.0
	3	79.5	79.5	0.0
	4	—	—	—
	Average	80.75	80.75	0.0
Whole blood (about 70 per cent erythrocytes suspension by hematocrite)	1	174	167.5	−6.5
	2	167.5	159.0	−8.5
	3	172.5	168.0	−4.5
	4	164.5	161.5	−3.0
	Average	169.6	164.0	−5.6

effect for each sample, i.e., the difference between the ordinates of the upper curves. The curves show that the flow effect increased as the concentration of the erythrocytes in the suspension was increased. The magnitude of the increase was not linear; this was also true when the

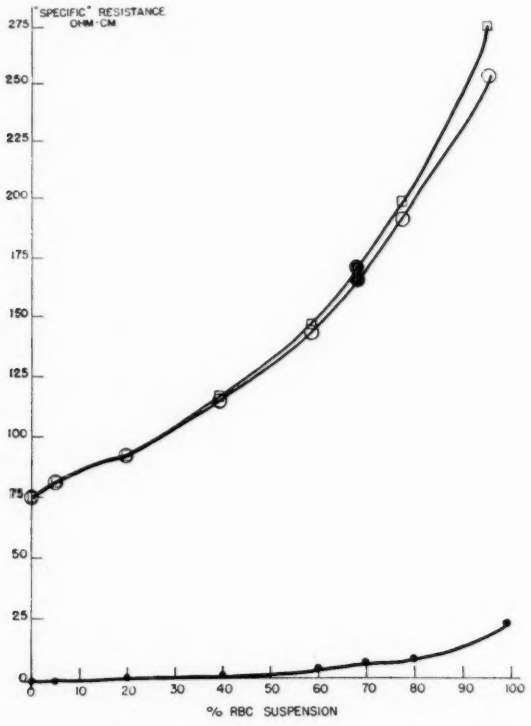

Fig. 2. Curve relating average "specific" resistance (of four different samples) of erythrocyte suspensions to concentration of the suspensions. Upper curve and open squares, average values at rest; middle curve and open circles, average values when in motion at linear velocity of 15 cm/sec.; lower curve, average values of flow effect (difference between ordinates of upper two curves). Solid circles show the average erythrocyte concentration of the whole blood as calculated from their average "specific" resistance values at rest and in motion. Discussed in text.

percentage change in resistance caused by the motion was calculated from the above data. When the electrical resistance of resting and moving erythrocyte suspensions is plotted as a function of the erythrocyte concentration, one can conversely try to determine the concentration of erythrocytes in the original blood from its resting and flow resistance.

The value obtained was about 70 per cent (cf. fig. 2) which checks satisfactorily with the hematocrite determinations.

The electrolytic composition of the medium in which the erythrocytes are suspended also has an effect on the magnitude of the flow effect. Some preliminary experiments, in which isotonic solutions of sodium, potassium, and calcium chlorides and of dextrose were used in equal proportions to dilute the erythrocytes, gave different flow effects, depending on the solution used.

A disproportionately large flow effect occurred in one sample of whole blood with a high resting electrical resistance, indicating a relationship between resting resistance and flow effect. It seems that it is due to the increased "specific" resistance or the increase of the total amount of erythrocytes in motion, as the introduction of a high series resistance in other blood samples did not increase the flow effect.

Some observations bearing on the mechanism of the flow effect. Our first assumption was that the decrease of resistance with flow might be due to an orientation of the erythrocytes so as to place their diameters along the line of flow and their concave surfaces facing at right angles to the line of flow. If this were true, one should find a concomitant increase in resistance if measured across the direction of flow. To test this possibility, a wide, flat, bakelite flow tube was constructed, 6 cm. x 7 cm. x 3 mm., and two pairs of electrodes were inserted, one pair in a line along the direction of flow, and the other pair in a line at right angles to the direction of flow. The electrodes of each pair were the same distance apart, 53 mm. The results of the resistance measurements with this tube are given in table 4.

It can be seen from this table that a decrease in resistance of the same order of magnitude occurred both along and across the line of flow. The fact that the transverse effect is somewhat smaller than the longitudinal effect is easily accounted for by the fact that the current in the transverse direction has to pass through portions of blood near the edges of the tube where the velocity of flow is less than in the center, whereas the axial current passes only through a region of maximum flow velocity. This result eliminates the possibility of orientation playing any rôle in the causation of the flow effect. Two other facts support this conclusion: 1. When two electrodes are immersed a fixed distance apart in a beaker of blood, transitory decreases in resistance are found when the blood is agitated. It is hard to conceive of any systematic orientation of erythrocytes taking place under these conditions. 2. A suspension of sawdust in water, when passed through the flow tube and examined under a strong light, showed definitely turbulent stream-lines at the velocities used in our experiments. No longitudinal orientation of erythrocytes could take place in such a turbulent stream.

Another possibility was tested, namely, that the flow effect was due to the creation of a more uniform distribution of the erythrocytes caused by agitation which would explain the effect of the flow through tubes in the presence of strong eddies. It is known that erythrocytes at rest tend not only to settle out of the suspension but also to associate in rouleaux formations. This state of the cells will tend 1, to reduce the effective serum volume available for electrocytic conduction of the electrical current by trapping serum between clumped erythrocytes; 2, to reduce the total effective surface area of the erythrocytes and hence the adsorptive properties, and 3, to decrease the number of isolated particles in the suspension. At the same time, the average size of the suspended particles will increase. Flow and agitation will tend to reverse these tendencies; it will "declump" the erythrocytes, so to speak, thus restoring the properties pertaining to a suspension of isolated erythrocytes. Which one of the three mentioned factors plays the deciding rôle in the observed effect

TABLE 4

	RESISTANCE AT REST	RESISTANCE AT FLOW OF 35 CC./SEC.	FLOW EFFECT
	ohms	*ohms*	*ohms*
Along flow	480	450	30
Across flow	510	490	20
Across flow	510	490	20
Along flow	480	450	30
Compensating "blood impedance" readjusted			
Along flow	460	430	30
Across flow	490	470	20

we have been unable to decide from our experiments. Two types of experiments were done which support this explanation. In the first, the electrical resistance of a sample of blood was measured in a large centrifuge tube after separation of the serum and erythrocytes by rapid centrifuging, and this measurement was compared with the resistance occurring after various degrees of remixing and "declumping" as effected by gentle shaking of the tube. The usual procedure with alternating current was followed. The two electrodes used were inserted in the centrifuge tube a fixed distance apart, one near the top and the other near the bottom of the tube. The results of a typical test are given in table 5. The values in table 5 could account for even greater changes in resistance than those actually observed as flow effects.

This experiment shows that making the suspension more uniform does decrease the electrical resistance and suggests further that whole blood may be more completely "declumped" by vigorous shaking. Similar

transitory changes in resistance were obtained when blood was agitated in a beaker in which measuring electrodes were immersed at a fixed distance apart. In line with this observation also is the fact that in our flow experiments we found that there is a variable time lag in restoring the resting resistance and in establishing the flow value of the resistance which would go hand in hand with a "declumping." A typical example

TABLE 5

	RESISTANCE
	ohms
Whole blood (control)	250
After centrifuging for 1 hour at high speed	435
After first shaking	305
After more shaking	290
After still more shaking	260
After very vigorous shaking*	200

* Further shaking caused no further decrease of resistance.

TABLE 6

	TIME	RESISTANCE	STATE OF BLOOD
		ohms	
Sample I. Crudely filtered whole beef blood	11:41	3726	At rest
	11:43	3270	Flowing at rate of 9.6 cc./sec.
	11:44	3553	At rest
	11:45	3600	At rest
	11:46	3640	At rest
	11:47	3660	At rest
	11:48	3680	At rest
	11:49	3720	At rest
	12:09	3700	At rest
Sample II. Well filtered whole beef blood	3:07	3770	At rest
	3:10	3516	Flowing at rate of 7.5 cc./sec.
	3:11½	3740	At rest
	3:12	3760	At rest
	3:13	3770	At rest
	3:24	3760	At rest

is shown in table 6. According to this view, the resting value corresponds to slightly clumped blood which is declumped by flow or agitation. One should anticipate that with increasing declumping a lower value of resistance should be approached, the minimum being in the state where all erythrocytes are separated. Indeed our curves approach a horizontal level at fast flows corresponding to this "saturation point."

The second experiment consisted of testing the flow effect in a sample of partially agglutinated blood and in a comparable sample of untreated blood. It was anticipated that the agglutination would cut down the "declumping" action of flow by making the adherence of erythrocytes stronger and so reduce the flow effect. The measurements were made with alternating current as in the original experiments. The inflow tube to the measuring tube was kept at a constant diameter so that the rate of flow in the two samples would be the same. For this experiment blood was obtained from a dog whose erythrocytes were found to be agglutinated by human convalescent serum (obtained through the courtesy of Dr. S. O. Levinson, in charge of the Michael Reese Hospital Serum Center). The defibrinated dog's blood was centrifuged at high speed and the erythrocytes and serum separated. One part of the dog's erythrocytes was suspended in an equal volume of the same dog's serum, and the other part of the dog's erythrocytes was suspended in an equal volume of an agglutinating mixture of human and dog's serum. This

TABLE 7

	RESISTANCE AT REST	RESISTANCE AT FLOW	FLOW EFFECT	RATE OF FLOW
	ohms	*ohms*	*ohms*	*cc./sec.*
Dog's erythrocytes suspended in an equal part by volume of dog's serum (no agglutination)	978	927	51	33.2
Same dog's erythrocytes suspended in an equal part by volume of human and dog's serum (partial agglutination)	964	938	28	33.7

proportion of dog's and human serum was found by microscopic examination to cause partial agglutination of the erythrocytes into clumps consisting of 2 to 5 erythrocytes not in rouleaux formation. The results are shown in table 7 and show a definite difference. Since the two samples were of the same concentration, a difference in dilution is ruled out. The adherence of agglutinated erythrocytes is more stable than that of the rouleaux in normal blood, hence our results could be interpreted as a lowered tendency to declumping of stable agglutinated erythrocyte conglomerates. Thus, the flow effect is apparently peculiar to blood because of the singular property of "stickiness" of its erythrocytes.

SUMMARY

It was found that the electrical resistance of defibrinated beef blood and dog's blood varies with the velocity of flow when the blood is put in motion. The tendency for the resistance to increase at slow flows is an "electrode effect" depending on the interaction between some samples of

blood and some types of electrodes. The decrease in resistance at faster flows is the true "flow effect." The change is primarily one of pure ohmic resistance, as shown by the fact that it occurs when either alternating or direct current is used for making the conductivity measurements. The experiments here reported show that the flow effect is peculiar to blood and is due to the presence of the formed red blood cells in the flowing stream. The experiments further indicate that the property of the erythrocytes to stick together in clumps which are subsequently separated by the flow motion is responsible for the resistance changes.

We are indebted to Dean H. G. Gale and Prof. J. B. Hoag of the Department of Physics, University of Chicago, for their courtesy in lending us the apparatus used in making the measurements.

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EXTIRPATION OF THE NEOCEREBELLAR CORTEX WITHOUT ELICITING SO-CALLED CEREBELLAR SIGNS[1]

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A previous investigation centered primarily upon the rubrospinal tracts in the monkey (1) resulted in our observing that a brachium conjunctivum was not essential for muscular stability either at rest or during movement. We subsequently investigated the effects of sectioning the brachia conjunctiva bilaterally in the dog (2) and found that this could be accomplished without causing any detectable disturbance. Accordingly, the question arose as to whether the absence of symptoms following this procedure was to be explained on the basis of the neocerebellum as a structural unit being silent in respect to the so-called cerebellar signs, or on the basis of its having another outflow in addition to that over the superior peduncles.

It is unmistakable that the disturbance in muscular movement which occurs following cerebellar injury has been interpreted, by many, as being due, at least in part, to neocerebellar deficit. The fundamental basis for this concept is the striking anatomical association of the neocerebellum with the cerebral hemispheres plus the clinical and experimental observation that, following cerebellar injury, it is voluntary movement which is most prominently disturbed. The concept has of recent years gained momentum as a result of the observation (3) that, in instances following decerebellization, reflex movements are executed with no detectable disturbance, as well as the demonstration that certain cerebellar signs disappear following subsequent removal of the cerebral hemispheres (4) or the excitable areas (motor) of the cerebral cortex (5).

Dysfunctions encountered following extirpation experiments on laboratory animals and accidental injury in the human have not suggested that neocerebellar function is any different from paleocerebellar function, but rather has supported the concept of the cerebellum "acting as a whole" (6) (7). However, active symptoms obtained from ablation experiments on the lateral lobes have been interpreted as being due to the extirpation.

[1] Aided by a grant from the Rockefeller Foundation.

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Clinically, disturbances have been encountered following lesions apparently confined to the neocerebellar cortex (10), while symptoms following tumors (7) (8) and atrophies of the cerebellum (9) have been suspected as being due to a deficient of the outflow over the superior peduncles; thus, the syndrome of the lateral lobes has been recognized as a separate entity from the syndrome of the vermis (11). In the dog and monkey delimited ablations of the neocortex have evoked characteristic disturbances in limb functions (12), while "extensive removals of the lateral lobes have in most instances precipitated on the homolateral side the typical pattern of total decerebellization except for the absence of hypertonus or the dynamic phenomena of Luciani" (13).

On the other hand, it is to be stressed that it has been "known since Flourens that a considerable portion of the cerebellar cortex can be removed without entailing clear or obvious symptoms" (14). Nothnagel (15) reports "destroying the majority of one or both hemispheres without the animal evincing any results for whole days" (rabbit). It is likewise recognized that lateral lobe tumors in the human frequently, if not usually, become massive before signs appear (11). Also severe symptoms clear rapidly and to a surprising degree subsequent to the successful removal of lateral lobe tumors and during the subacute state following experimental or accidental lesions. These negative results seem, without exception, to have been explained on the basis of the ability of the remaining cortex to function adequately for the portion ablated.

METHODS. *Terminology.* In speaking of the neocerebellar cortex we have adopted the usage of Ingvar; this includes the whole of the middle lobe which consists of the lunate, ansiformis, paramedian and tuber lobules. Because we were particularly interested in determining the effects of removing the total pontine inflow into the cerebellum, care was taken in certain experiments to remove, in addition, the lobule paraflocculus because of the prominence of the pontine inflow into its cortex. This inflow was readily demonstrated by gross dissection, on formalin-fixed material, of the pontine bundle upward into the cerebellar cortex. Although it is certain from such a dissection and from retrograde degeneration following cortical ablations (see fig. 4*a* and *b*) that there is also a rich inflow of pontine fibers into the median cortex of the posterior and anterior lobes, removal of the above-mentioned lobules with underlying damage has, in instances, sectioned all pontine fibers as evidenced by the complete retrograde degeneration of the pontine bundle and nuclei (see fig. 1*a*).

Animals. Both the dog and monkey were selected because better interpretations can be obtained of the disturbances in postural functions in the dog than in the monkey; and because better interpretations can be obtained of disturbances in the so-called "voluntary movements" in the monkey than in the dog.

Operative procedures. In the dog, the bone covering the cerebellum was exposed

by 1, separating the dorsal and caudal connections of the temporal muscle and retracting it outward; 2, dividing the dorsal cervical muscles in the midline, retracting them, and, on the side of operation, cutting them free from the occipital crest. Hemostasis was obtained by clamping the muscles with a long bladed hemostat prior to their severance. The bone was then removed piecemeal with rongeurs, working caudad to the magnum foramen from a trephine hole drilled directly over the occipital pole. The dura over the cerebral hemisphere was never punctured but was elevated slightly upward from the bony tentorium. The bony tentorium in turn was removed, a procedure which is necessary in order to visualize adequately the anterior margins of the lunate and paraflocculus lobules. In doing this great caution must be exercised both at the medial and the lateral attachments of the tentorium to avoid opening the large venous sinuses located at these positions. The dura was then divided and retracted, thus exposing the whole cortex of the lateral lobe. Following exposure, the cerebellar cortex was removed by 1, cauterization of all surface blood vessels, and then 2, careful separation of each lobule, from adjacent lobules, along its bordering sulci, after which 3, the isolated lobule was pinched free at its base with fine blunt forceps. This method allows for accurate, specific demarcation of tissues removed, with complete hemostasis, and without infringement upon neighboring structures. It is important that the dog be completely relaxed by full anesthesia (pentobarbital sodium) throughout the whole procedure, *including the closure*, to avoid straining of any kind which is so prone to elicit hemorrhage from the unavoidably exposed sinuses.

In the monkey, the exposure of the cortex was made by turning down a large bone flap so as to expose the caudal portion of the cerebral hemisphere on the side of operation. This opening was then extended, with rongeurs, caudad, over the surface of the cerebellum, to the magnum foramen. In turn, the occipital lobe was retracted upward and outward, thus exposing the dura covering the lateral one-half of the cerebellum. The cortex was then exposed by lateral retraction of the dura, which includes the tentorium, after cutting it along its medial attachments. This exposure visualized the cerebellar cortex from the midline laterally, except for that of the paraflocculus, which was in turn brought into view with only slight manipulation. The removal of the cortex was accomplished, as in the dog, with complete hemostasis by surface cauterizing, lobule isolation along sulci and pinching free at the base with blunt forceps.

Tissue. At the termination of the experiments the brains were perfused with 10 per cent neutral formalin, removed, and blocked. After photographing, the cerebellar block was sectioned serially in paraffin and appropriate sections were stained for cells and fiber tracts.

RESULTS. *Unilateral extirpations in dog.* In a series of 30 dogs unilateral ablations were performed, ranging from individual lobules to all of the lunate, tuber ansiformis, paramedian and paraflocculus lobules, collectively. Symptoms encountered in turn ranged from the maximal described to follow hemi-decerebellization to essentially no immediate or subsequent deviation from the normal. So long as there was no gross infringement upon the medial cerebellar nuclei or the underlying vestibular nuclei, neither the character, severity nor duration of the symptoms could be correlated in any way with the location or extent of the extirpation. The symptoms appeared, however, to be due to factors associated with edema, hemorrhage and infection. The ease with which an animal that

was essentially symptomless, during the first few days following operation, would suddenly exhibit severe symptoms and the remarkable rate and degree of recovery from symptoms when present were striking.

The removal of the left lateral lobe complex—lateral extension of the lunate, ansiformis, paramedian and paraflocculus lobules—is evidenced in the photograph of the cerebellum of dog 1 shown in figure 2*a*. Routine histological studies demonstrated removal of the bulk of the lateral cerebellar nuclei with no infringement upon the medial nuclei or the prominent fiber tract which takes origin from vicinity of the latter and courses direct to the underlying medulla. The section shown in figure 1*a* is taken from the series on this dog and evidences the retrograde degeneration of the brachium pontis which was complete.[3]

Following operation, dog 1 showed no deviation from the normal except that, when walking, the left feet were raised slightly higher off the floor than the right feet. This asymmetry, though definitely present, was barely perceptible, and was present only for the first few days after operation. He righted normally from both sides directly and walked with no evidence of staggering. When the feet were passively placed on their dorsa, or the legs pulled laterally or backward into awkward positions, the dogs immediately corrected their abnormal position except for an occasional slight delay in correcting the left rear foot. There was no detectable asymmetry in muscle tone or disturbance in forward or backward balance. The dog, post-operative, daily stood erect on its rear legs for food with no evidence of asymmetry and on the 3rd day actually walked forward in this position.

The photograph shown in figure 1*b* is a positive Marchi showing complete degeneration of a brachium conjunctivum in dog 2. The cortex extirpated in this dog was identical with that of dog 1 except that there was greater completeness in removal of the lateral cerebellar nuclei. This dog likewise exhibited no deviation from the normal in any respect except for the over-raising of the left foreleg when walking, which was more marked than was the case of dog 1. This deviation cleared progressively, having entirely disappeared by the 7th day.

In other dogs, the medial portion of the lunate lobule and the tuber were removed in addition to the lateral lobe without precipitating any distinct dysfunction. Likewise, in instances, involvement of the lateral cerebellar nuclei to the extent of that in dog 2 was accomplished without eliciting any detectable over-raising of the homolateral limbs.

Moderate or severe symptoms appeared immediately after the operation very infrequently in the entire series. Usually when they occurred, symptoms were anticipated at the time of closure because of indication of

[3] This dog was demonstrated in motion pictures before the American Physiological Society in Detroit in 1935.

edema of the cerebellar tissue underlying the ablation. Edema is exceedingly prone to appear if hemostasis is not sufficiently controlled during the process of cortical extirpation. In two instances, immediate symptoms were undoubtedly due to post-closure hemorrhage which resulted because of the animal straining during light anesthesia. On the other hand, the onset of severe or moderate symptoms, some days after operation, occurred rather frequently and as a rule were precipitated suddenly by *a*, pressure applied intentionally or accidentally over the region of bony defect, or *b*, from an infected wound. In one animal with a left sided ablation, the mere palpation of the swollen area over the bony defect precipitated violent rotation for several seconds, following which the animal lay prostrate on its right side unable to move without eliciting another seizure of

Fig. 1. *a*. Section from the series on dog 1. The degenerated brachium pontis on the left is evident. Note that the medial portion of the lateral nuclei remains on the left at this level.

b. A section from dog 2 stained with osmic acid. Note the degenerating ascending and descending components of the left brachium conjunctivum.

rotation. After operation, up to the time of the accident, the animal was entirely normal except for a slight over-raising of the left limbs. Another dog was suddenly put off his feet as a result of bumping his head on the cage when he withdrew his head suddenly from the hands of the observer as the head was being examined for suspected infection. In several instances, in dogs essentially symptomless following operation, the mere release of the pressure caused by pus in the field of operation precipitated typical attacks of rotation. In still other instances, the post-operation precipitation of symptoms was clearly due to post-operative hemorrhage.

Symptoms of the so-called cerebellar type encountered in the series can be classified on a functional basis as follows:

1. Over-raising of the limbs on the side of the lesion when walking. Usually the

lifting of the limb was more pronounced in the fore leg than in the rear leg although the exact opposite was observed in a few instances. The amount of over-raising varied from a just perceptible deviation to that of almost complete flexion at all joints. It is noteworthy that marked flexion was at times present in the absence of any leaning or staggering to the affected side.

2. Failure to correct abnormal positions of the limbs. In its milder form this was evidenced as merely a delay in correction, whereas, in its more severe form, there was standing on the dorsa of the feet and dragging on the dorsa in walking. This dysfunction was likewise seen occasionally in the absence of leaning or staggering to the affected side.

3. Postural defects: *a*. Leaning with the head and body either to the side of operation or to the opposite side, when standing with the opposite limbs extended laterally in a sort of grasping attitude. Such an animal when walking staggered or fell to the side to which the body leaned. Staggering occurred frequently when walking, even when there was no asymmetry in the standing posture.

b. In mild cases, when the animal fell on to its side, it either 1, remained on this side at rest; 2, righted again to its feet indirectly, or with some modified form of indirect righting; or 3, rolled over on its back to the opposite side, i.e., legs rotating in the air. From the opposite side the animal righted immediately to its feet directly in a hyperactive manner.

c. In severe cases, the sequence of events listed in *b* occurred in rapid succession resulting in frequent rapid rotation (vestibular fit). When rotation ceased, the animal always came to rest on the side to which it fell from the standing position.

Staggering and falling with rotation occurred in a direction opposite to the side of ablation as well as to the same side (8). The former occurred in higher incidence than the latter. The contralateral symptoms could not be explained on the basis of anatomical involvement of the cerebellar or vestibular structures on the opposite side. That mechanisms for both contralateral and ipsilateral derangements in postural reactions are located at the general level of the vestibular nuclei on each side is evident because hemisection of the brainstem at any location below the level of the cerebellar peduncles invariably resulted in ipsilateral impaired righting, whereas hemisection of the brainstem through the pons or midbrain resulted in contralateral impairment of the same nature. The area of transition between the two effects was, therefore, at the general level of the cerebellar peduncles.

4. A tendency for the animal to fall backwards or forwards. When this dysfunction was present following unilateral lesions it was usually asymmetrical, as shown by the animal's body falling towards the side of the lesion while going in the forward or backward direction.

Bilateral ablation in the dog. The absence of dysfunction following unilateral removal of the neocerebellar cortex with the bulk of the underlying lateral nuclei, raised the question as to whether the remaining contralateral neocerebellar cortex might not exert a crossed control as well as an ipsilateral control over the body musculature. If this were true, removal of the remaining neocerebellar cortex at a date subsequent to the first removal should not only precipitate both ipsilateral and contralateral signs but should also give the complete neocerebellar extirpation deficit syndrome, if any.

That bilateral ablation of the neocerebellum can be accomplished in the dog without eliciting cerebellar signs is evident from dogs 3 and 4. In dog

3, both lateral lobes including the paraflocculus lobules were successfully removed in two operations. The vermian neocerebellar cortex was not extirpated. Following the removal of the cortex on the left (1st operation) the animal showed no deviation from the normal. On the 7th day after the 1st operation, in demonstrating this dog along with others to students, its head was bumped by another dog. This bump elicited a sudden tipping of the head to the right with staggering to the right. The dog did not fall but reached a wall against which it leaned. Fifteen minutes later the dog was again normal. Following the 2nd operation—removal of the right lateral lobe—the dog again showed no detectable deviation from the normal. After four weeks in the cage, for protection while the head was being

Fig. 2. *a*. Dorsal view of the cerebellum of dog 1 which evidences the complete removal of the left lateral lobe and in addition illustrates well the division of the organ into lobes and lobules.

b. Caudal view of the cerebellum of dog 4 which evidences removal of the lateral lobes—including the paraflocculus lobules—and involvement of the cortex of the tuber and medial portion of the lunate lobule.

healed and accordingly stabilized, the dog was placed in the stockyard and, on the basis of motor activity, could not be detected from the normal dogs.

The photographs in figure 2*b* and figure 4 show the cerebellum of dog 4[4] in which essentially all of the vermian neocerebellar cortex was removed in addition to both lateral lobes. The vermian cortex was removed at the first operation when the left lateral lobe was ablated. Following the 1st operation, there was a slight over-raising of the left fore leg which lasted for a few days. The dog, in spite of being large and adipose, stood erect on its rear feet for food with no evidence of asymmetry. Following the second operation there was no deviation from the normal except on three

[4] This dog was demonstrated in motion pictures before the American Physiological Society in Washington 1936.

occasions slight over-raising of the right limbs occurred suddenly as a result of the dog bumping its head. At these times there was no evidence of any deviation from the normal in the left limbs. The dog was terminated three weeks after the 2nd operation. Marchi staining of a block of tissue from the midbrain at the level of the red nuclei demonstrated prominent degeneration of both brachia conjunctiva.

In dogs where moderate or severe symptoms appeared subsequent to the 2nd operation, such symptoms were identical to those described above following unilateral removals (i.e., 1st operation). There was no evidence of contralateral involvement even in the presence of severe ipsilateral signs.

Ablations in monkeys. The lateral extension of the lunate, and the whole of the ansiformis, paramedian and paraflocculus lobules were removed on the left side in monkey 1. There was no great infringement upon the underlying dentate nucleus. Ten hours after administering the anesthetic the monkey was moving about the cage actively with no evidence of disturbance in posture. Careful examination, by passive manipulation failed to detect any asymmetry or deviation from the normal in muscle tone. He fed readily with both hands, there being no deviation from the normal in the right hand, but there was an occasional definite but slight over-reaching of the left hand present when picking up food and in placing it to its mouth. On the second day, in addition to over-reaching, there was a slight postural tremor and a moderate spontaneous tremor present in the left arm. Both tremors were present only periodically. All symptoms were still present but just barely detectable on the 3rd day and were never observed on or subsequent to the 4th day.

In monkey 2, in addition to the left lateral lobe, the vermian portion of the lunate and the tuber were removed. Subsequent to operation this monkey showed no deviation from the normal in any respect. On the 1st and 2nd day after operation he not only walked erect on all fours but walked across the cage erect on the rear feet holding half an orange in his hands.

During the procedure of removing the neocerebellar cortex on the left side, in monkey 3,[5] because of temporary disorientation, the region of entrance of the vestibular nerve (into medulla) was encroached upon. The morning following operation, the monkey was sprawled on the bottom of the cage on its abdomen with its limbs out laterally in a grasping attitude. In addition the whole body leaned abruptly to the right side and the head was tipped and rotated so that the dorsum of the head was to the right and the chin to the left. Rotary nystagmus was prominent. In the late afternoon the monkey was sitting almost erect in the cage though there was still definite leaning to the right. In this position the monkey fed readily reaching for small bits of banana with each hand, and manipu-

[5] This monkey was demonstrated in motion pictures before the American Physiological Society in Washington 1936.

lated half an orange with both hands, showing no evidence of dysfunction. Subsequently the monkey's posture returned to normal rapidly and pro-

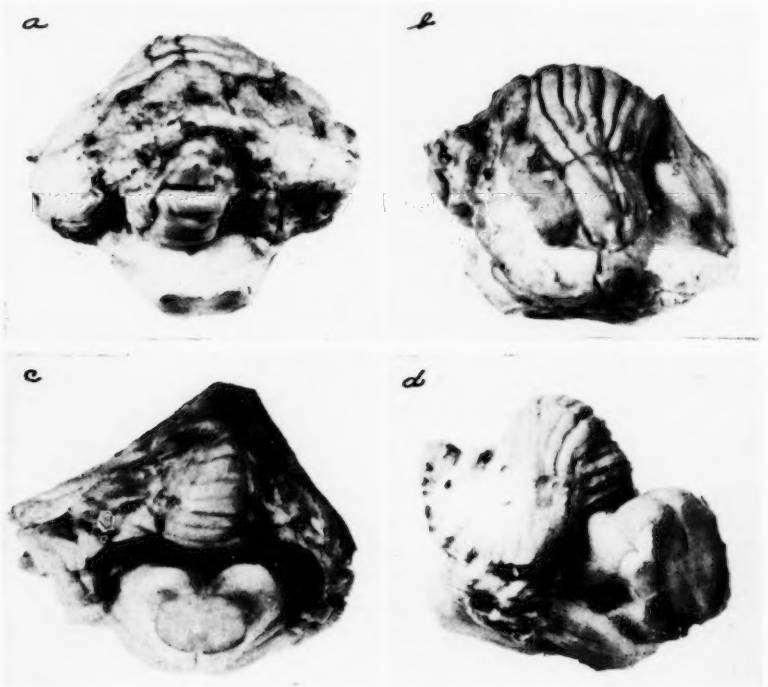

Fig. 3. *a*. Caudal view of the cerebellum of monkey 3 which evidences removal of the cortex of the middle lobe of the cerebellum and in addition the cortex of the paraflocculus lobules without infringement upon the medial cortex of the posterior lobe.

b. Right lateral view of the cerebellum of monkey 3 which better demonstrates the extent of the lateral removal as well as the intactness of the anterior lobe.

c. Caudal view of the cerebellum of monkey 4. Note that the cortex of the posterior lobe is undisturbed on the left side as well as medially whereas the lobules paraflocculus and flocculus are decorticated on the right side.

d. Right lateral view of the cerebellum of monkey 4 which further evidences the removal of the floccular lobules on this side. Compare the depth of removal of the medial cortex in this specimen with that in monkey 3 shown in *b*. Also note that the cortex of the culmen lobule of the anterior lobe is encroached upon.

gressively over a period of several days. There was no evidence at any time, however, of dysfunction in voluntary movements, nor was any tremor present.

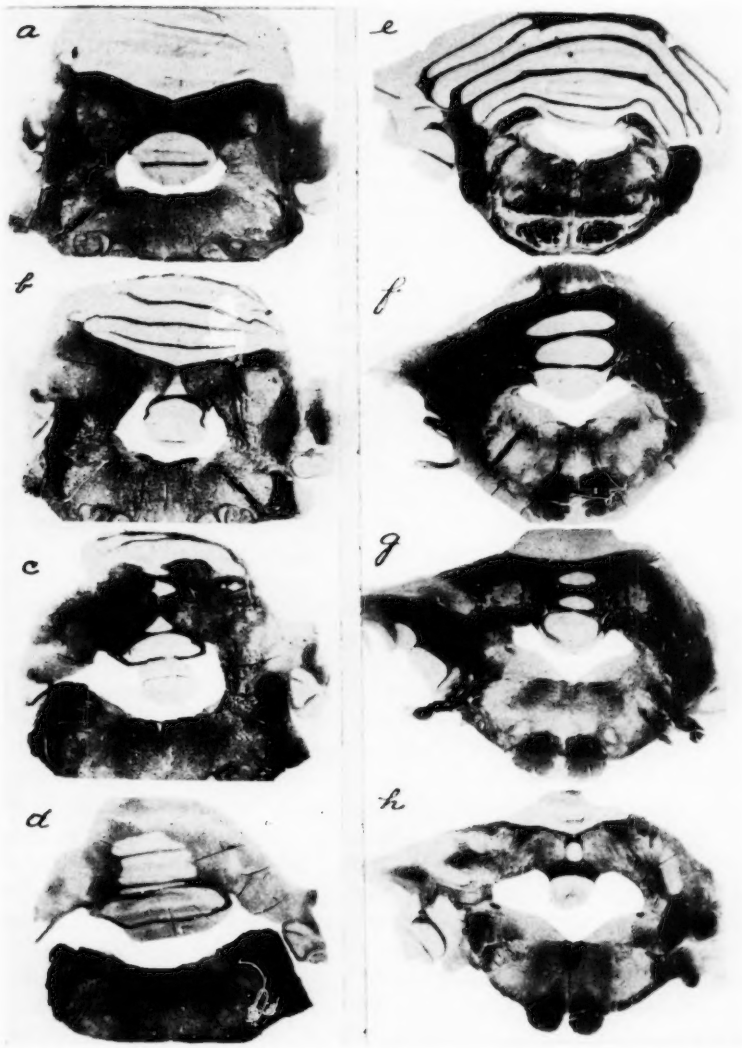

Fig. 4. Representative photomicrographs of sections stained for fiber tracts taken from the series on dog 4 (*a*, *b*, *c* and *d*) and monkey 4 (*e*, *f*, *g* and *h*). In dog 4 note the intactness of the medial nuclei and the fibers passing from their vicinity to the underlying medulla. Also that the medial portions of the lateral nuclei remain.

In monkey 4 note that the lateral nuclei are not infringed upon on the left side but that the dentate nucleus is largely eliminated on the right side. Particularly noteworthy is the selective retrograde degeneration of the pontine brachia. Compare the pontine brachia in sections *e* and *f*.

At a subsequent operation, the remaining neocerebellar cortex (right), including the paraflocculus lobule, was removed, except for remnants of the deeper folia of the vermian portion, as evidenced by the photograph, figure 3*a* and *b*. The monkey, following operation, was up and about its cage actively with no detectable dysfunction either in the right or left side of the body. The only deviation from the normal that could be detected in this monkey subsequent to the 2nd operation was a preference for using the left hand in feeding. This appeared on the 5th day and lasted for 3 or 4 days. During this period of preference for the use of the left hand, the right hand was used readily at times with no detectable disturbance.

In monkey 4, care was taken to make certain that all the neocerebellar cortex medially and laterally was extirpated because in monkey 3 remnants of the deeper vermian folia remained. The neovermian cortex was removed with the cortex of the lateral extension of the middle lobe on the left side. The paraflocculus was not removed on the left side but was completely removed on the right side as is evidenced by the photograph in figure 3*c* and *d* and figure 4. In addition to removal of the neocerebellar cortex the adjacent cortex of the anterior and posterior lobes was encroached upon. In spite of this encroachment the animal subsequent to each operation exhibited no asymmetry or dysfunction in posture or muscle tone. For a day or two after each operation there was an occasional, just perceptible, lack of smoothness, in placing food to the mouth, in the hand on the side of operation. Likewise, several days subsequent to each operation, a postural tremor appeared as a result of holding a heavy piece of food, such as an orange, in the hand on the side of operation. These symptoms, though present, were barely detectable and cleared rapidly. There was no evidence of contralateral symptoms following either operation.

Discussion. All theories of cerebellar function have been formulated largely on the basis of symptoms manifested in the presence of experimental, accidental or pathological injury to the cerebellum. Therefore, since the whole of the neocerebellar cortex as well as at least the greater bulk of the underlying neocerebellar nuclei have been successfully ablated without eliciting even transitorily so-called cerebellar signs it seems justifiable to conclude that the *neocerebellum is not essential for any of the functions that have been attributed to the cerebellum.* Although this statement of fact does not in itself deny that the neocerebellum mediates any such functions, it questions that possibility. If the neocerebellar cortex does for instance mediate a control over voluntary muscular movement to the degree previously assumed, the absence of disturbance following its removal must be explained on the basis of *immediate* compensation by other structures. One naturally thinks of the remaining cortex of the anterior and posterior lobes as the possible compensating tissue, particularly in the light of the

uniform structure of the cerebellar cortex which suggests a unitary function. It is difficult, however, to conceive that this situation exists in view of the removal of the pontine inflow into the cerebellar cortex without precipitating dysfunction.

In order to test the possibility of compensation by the vermis, extirpations of the vermis—cortex and nuclei—were performed leaving the lateral lobes, lateral cerebellar nuclei, and the brachia conjunctiva intact. In the dog this procedure precipitated the essential pattern of total decerebellization as given by Dusser de Barenne and Rademaker with the exception that there was no spontaneous extensor rigidity and the final state was reached more quickly. The deficit seemed to be fundamentally postural in nature, being a combination of a disturbance in balance and an exaggerated standing reflex. In the monkey both the immediate and residual deficits following removal of the vermis were likewise postural in nature and equivalent to that seen in the dog although not quite so prominent. Disturbances in muscular control as evidenced by ataxia during feeding movements and by the presence of static tremor were slight and entirely transitory. Removal of the left lateral lobe, completely, with its underlying nuclei, in addition to the vermis, did not intensify either the transitory or residual symptoms of vermian ablation, in the monkey, thus indicating that there is no apparent compensation by the vermis for the lateral lobes following their removal or vice versa.

An analysis of a few preliminary experiments from a comprehensive decortication series—anterior and posterior lobes individually, total vermis and total cerebellum—indicate that the whole of the cerebellar cortex is actually silent, with respect to its ablation, precipitating cerebellar signs. Accordingly, the question is raised as to whether the entire cerebellar cortex with the lateral cerebellar nuclei and their outflows over the superior peduncles should not be considered as making up the cerebellar organ proper—the function of which remains to be determined—and whether the roof nuclei with the direct cerebello-bulbar tracts should not be considered primarily as a portion of the vestibular complex (postural). Anatomical support for such a functional division is afforded by 1, the uniform structure of the cerebellar cortex; 2, the fact that the medial cerebellar nuclei receive direct afferent fibers other than from the cerebellar cortex, whereas the lateral cerebellar nuclei do not (17), and 3, the fact that the pontine fibers are distributed to the whole of the cerebellar cortex.

The abrupt post-operative precipitation of typical severe cerebellar and related signs in the dog and the post-operative appearance and intensification of tremor and ataxia in the monkey with their subsequent rapid and complete clearing demonstrates the ease with which neighboring structures can be temporarily deranged by factors that are not related to any deprivation effect of the tissue previously extirpated. Accordingly, we are prone to think of the clearing of symptoms which followed immediately after

the operation as being due to the return to normal function of structures temporarily affected by the operative procedures rather than to compensation by related or subsidiary structures.

Whether the absence of symptoms and the rapid clearing of symptoms, when present, are to be explained on the basis of immediate and rapid compensation or on the basis of freedom from encroachment or temporary impairment of neighboring structures, or both, it seems that we must look to the neighboring brainstem in the last analysis for the actual structures whose derangement is responsible for the so-called clinical cerebellar signs, the only exception being the postural deficit which seems to be associated with degeneration of the cerebello-bulbar tracts. It is pointed out that the irregularities in muscular movement as well as the tremors were of greater severity and longer duration—apparently permanent—in two monkeys (monkeys 10[6] and 9) of our original series (1) with brainstem lesions than that seen in our present series where the cerebellum was approached directly. In addition, our own experimental series have in instances verified and in others demonstrated that 1, awkward limb positions (1) (2); 2, over-raising of unilateral limbs (18); 3, spontaneous involuntary muscular movements (19); 4, atonia (20); 5, unilateral impairment of righting (21) are all entities showing permanent deficits following experimental lesions placed in the pontile portion of the brainstem.

Finally, unless it can be shown that neocerebellar function is related to a control over muscular movement such that its deprivation effects are not detected by clinical observation we are left without a ready lead from extirpation experiments for the elucidation of neocerebellar function (cerebellar function proper?). Certainly the most suggestive lead in either case is the anatomical one, namely, the close association of the cerebral hemisphere with the cerebellum by way of the cerebro-pontine fibers. Therefore, the recognition of the exact origin of the cerebro-pontine fibers in the cerebral cortex with the elucidation of the function of that portion or portions of this cortex would seem to be the most promising approach to this problem (5).

SUMMARY

Partial and complete ablations of the cortex of the middle lobe of the cerebellum—lunate, tuber, ansiformis, and paramedian lobules, and in addition the paraflocculus lobules—as well as the underlying lateral cerebellar nuclei were successfully performed in monkeys and dogs without eliciting detectable dysfunction.

[6] This monkey was demonstrated in motion pictures before the section on Neurology and Psychiatry of the Southern Medical Association in Richmond, Virginia in 1933, and also before the American Physiological Society in Detroit, 1935.

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THE EFFECT OF HEMORRHAGE, INTESTINAL TRAUMA AND HISTAMINE ON THE PARTITION OF THE BLOOD STREAM[1]

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A method for the direct measurement in unanesthetized dogs of the blood flow through the kidneys was devised recently by Mason, Blalock and Harrison (1936). A similar method for the liver was described by Blalock and Mason (1936). Levy and Blalock (1936) have adapted the method to the determination of the flow of blood from two additional regions of the body by measuring the flow of blood in the distal part of the inferior vena cava, coming largely from the posterior extremities, and the blood flow in the superior vena cava, coming mainly from the anterior extremities and head.

In the present experiments, the effects of hemorrhage, of trauma to the intestines and of the injection of histamine on the flow of blood from the kidneys, the portal system, the posterior part of the body and the anterior extremities and head have been determined.

METHOD AND RESULTS. Dogs were used in all experiments. They were trained to lie quietly upon the table. No anesthesia was used in most instances except for the injection of novocain in the region of the external jugular vein where a cannula was to be introduced. A few of the animals were given morphine prior to the beginning of the studies. In the three experiments in which the intestines were traumatized, ether anesthesia was administered following the completion of the control determinations. The animals exhibited no evidence of pain during the course of the experiments. Heparin was used in order to prevent clotting of the blood. The cardiac output determinations were performed by the use of the Fick principle. The arterial blood pressure was measured by the employment of needle puncture and a mercury manometer.

The effects of hemorrhage. Following the control determinations on the flow through the various regions in seven experiments, blood was allowed to escape from the cannula until the mean arterial blood pressure fell to levels varying from 80 to 100 mm. Hg. After intervals ranging from 5 to

[1] Aided by a grant to Vanderbilt University from the Division of Medical Sciences of the Rockefeller Foundation.

10 minutes, the flows were determined again. The average reduction in the flow through the posterior part of the body was 76 per cent, that for the kidneys was 48 per cent, the portal system 53 per cent and the flow of the anterior extremities and head was reduced 54 per cent. After the lapse of several minutes, the flows were determined again. In only one instance (exper. 1) was additional blood removed between these two sets of determinations. The average reduction in the flow through the posterior part of the body was 86 per cent, the kidneys 46 per cent, the portal 48 per cent and the anterior extremities and head 58 per cent. Following these studies, the output of the heart was determined. The total decrease in the flow of blood through the four regions was always considerably greater than the quantity of blood which had been removed. The results of these experiments are given in table 1.

TABLE 1

The effect of hemorrhage on blood flow

EXPER. NO.	WT., KGM.	CONTROL FLOWS, CC. PER MINUTE					BLED, CC.	B.P., MM. Hg	FLOWS AFTER BLEEDING, CC. PER MINUTE					B.P., MM. Hg	SUBSEQUENT FLOWS, CC. PER MINUTE					CARDIAC OUTPUT, CC. PER MINUTE	REMARKS
		Post. part	Kidneys	Portal	Ant. part	Total			Post. part	Kidneys	Portal	Ant. part	Total		Post. part	Kidneys	Portal	Ant. part	Total		
1	14.6	283	273	405	556	1517	300	95	60	135	259	242	596	65	38	98	246	176	568	543	Local
2	9.95	153	233	375	319	1080	250	95	14	83	96	113	306	95	17	115	99	102	333	341	Local
3	13.2	261	256	451	375	1343	275	80	13	94	234	217	558	80	5	120	263	170	558	615	Local
4	14.9	321	361	556	600	1838	450	90	68	120	221	250	659	60	32	163	273	243	711	632	Local
5	14.9	297	469	536	484	1786	500	95	62	238	175	224	699	90	42	208	242	242	734	735	Morphine
6	14.4	105	183	297	469	1054	330	100	61	124	161	238	584	100	35	183	208	242	668	559	Morphine
7	11.8	149	246	300			250	90	49	221	187										Morphine
Average.		224	289	417	467	1436			47	145	190	214	567		28	148	222	196	595	571	
Average per cent reduction.....									76	48	53	54			86	46	48	58			

The effects of intestinal trauma. Three experiments of this type were performed. The control determinations were carried out without anesthesia. Ether was then administered and most of the intestines were delivered through a midline abdominal incision. Trauma was produced by massaging the intestines for periods ranging from 30 to 43 minutes. The contents of the peritoneal cavity were replaced and the incision was closed. The blood pressure at the time of the subsequent studies varied from 75 to 85 mm. Hg. Determinations which were performed following closure of the incision showed that the flow through the posterior part of the body was reduced 73 per cent, the kidneys 24 per cent, the portal system 29 and the anterior extremities and head 39 per cent. In one experiment in which the pulse remained strong throughout the traumatization, almost the entire reduction in the flow took place in the anterior

and posterior portions of the body. The results of these experiments are given in table 2.

The effects of histamine administration. Five experiments of this type were performed. In two instances, histamine was administered intravenously and in three it was given subcutaneously. The blood pressures subsequently at the time of the measurement of the flows ranged from 50 to 85 mm. Hg. Shortly following the injection, the average reduction in the blood flow of the posterior part of the body was 44 per cent, the kidneys 36 per cent, the portal system 37 and the anterior extremities and head 47 per cent. Subsequent determinations revealed approximately the same alterations. However, there were greater individual variations in the results of these experiments than in those of the two groups in which hemorrhage and intestinal trauma were studied. The results of these experiments are given in table 3.

TABLE 2

The effect of intestinal trauma on the blood flow

EXPER. NO.	WT., KGM.	CONTROL FLOWS, CC. PER MINUTE					TRAUMA DURATION, MINUTES	B.P., MM. Hg	FLOWS AFTER TRAUMA, CC. PER MINUTE					B.P., MM. Hg	SUBSEQUENT FLOWS, CC. PER MINUTE					CARDIAC OUTPUT, CC. PER MINUTE
		Post. part	Kidneys	Portal	Ant. part	Total			Post. part	Kidneys	Portal	Ant. part	Total		Post. part	Kidneys	Portal	Ant. part	Total	
8	11.7	234	283	556	484	1557	31	75	55	172	312	259	798	80	57	185	313	262	818	623
9	13.1	224	333	577	577	1711	44	80	46	242	405	300	993	80	57	167	375	294	893	802
10	12.7	277	300	600	556	1733	42	85	107	288	517	429	1341	85	85	300	517	429	1331	1148
Average per cent reduction.....									73	24	29	39			73	28	31	39		

Discussion. Erlanger, Gesell and Gasser (1919) demonstrated an increase in the peripheral vascular tone in experimental shock. Gesell (1919) found that a decrease in blood volume of less than 10 per cent produced by hemorrhage may elicit through constriction of central origin a decreased flow of blood through the submaxillary gland of more than 60 per cent even though accompanied by a rise in pressure. Similarly it was observed that tissue abuse produced by manipulation of the intestines may produce a diminution in volume-flow equally as great. Freeman, Shaw and Snyder (1936) found that the blood flow through the hand is markedly reduced in patients who are in shock. In commenting upon the results of their experiments and others, they state, "These observations indicate that the reduced blood flow in the hand, observed in clinical cases of surgical shock, represents a deficiency which is probably general throughout the body." Our experiments on hemorrhage and tissue abuse show a marked diminution in the flow of blood through the extremities and a less striking decrease in flow elsewhere.

This would be expected in view of Gesell's studies on the submaxillary gland. It is interesting in the experiments on hemorrhage that the total decrease in flow through the four regions which were studied was considerably greater than the volume of blood which was removed.

The results of the experiments in which histamine was injected show greater individual variations than the others. Nevertheless, they indicate a response on the part of the circulation which is definitely different from that observed following hemorrhage and tissue abuse. The reduction in flow following the injection of histamine is more nearly uniform throughout the body. This is further evidence against the histamine theory of shock. Johnson and Blalock (1931) found that hemorrhage and trauma are associated with first a decline in the output of the heart

TABLE 3

The effect of histamine on the blood flow

EXPER. NO.	WT., KGM.	CONTROL FLOWS, CC. PER MINUTE					HIST., MGM.	B.P., MM. Hg	FLOWS AFTER HISTAMINE, CC. PER MINUTE					B.P., MM. Hg	FLOWS AFTER HISTAMINE, CC. PER MINUTE					CARDIAC OUTPUT, CC. PER MINUTE	REMARKS
		Post. part	Kidneys	Portal	Ant. part	Total			Post. part	Kidneys	Portal	Ant. part	Total		Post. part	Kidneys	Portal	Ant. Part	Total		
11	9.4	104	170	259	441	974	4	60	67	97	227	197	588	67	69	98	238	172	577	555	No anesthetic. Hist. subq.
12	8.8	126	316	469	395	1306	6	75	117	183	366	231	897	85	93	190	357	227	867	700	No anesthetic. Hist. subq.
13	13.4	230	283	600	556	1669	3.5	50	138	203	283	288	912							493	Morphine. Hist. intravenously
14	13.4	300	300	330	500	1430	2.5		81	161	147	238	627		73	106	102	169	450	356	No anesthetic. Hist. intrav.
15	11.8	299	326	577	536	1738	8	80	114	250	405	326	1095	80	66	283	405	300	1054	1155	No anesthetic. Hist. subq.
Average per cent reduction.....									44	36	37	47			52	40	33	54			

which is followed subsequently by a decline in the blood pressure. The alterations appear in the reverse order following the injection of histamine. Roome, Keith and Phemister (1933) produced a decline in the blood pressure to a shock level by a variety of procedures which included hemorrhage, tissue abuse and histamine injection. The quantity of blood that it was necessary to remove in order to cause death was then determined. The removal of a relatively small quantity caused death when the initial decline had been produced by hemorrhage or tissue abuse.

The cardiac output determinations which are reported here were performed at the conclusion of the studies. The values were usually slightly lower than the total of the flows through the four regions which were investigated. This is obviously wrong since the flow through at

least one organ, the heart, was not included in the fractionation. This discrepancy may be due to an alteration in the condition of the animals which took place between the two determinations.

SUMMARY

The flow of blood through the posterior part of the body, the kidneys, the portal system and the anterior extremities and head has been determined before and after, *a*, hemorrhage; *b*, intestinal trauma; and *c*, histamine administration. Hemorrhage and intestinal trauma were accompanied by a greater diminution in the flow of blood through the posterior part of the body and a smaller decrease elsewhere. The injection of histamine was usually associated with a general reduction in the flow of blood throughout the body.

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THE SIMULTANEOUS MEASUREMENT OF RENAL BLOOD FLOW AND THE EXCRETION OF HIPPURAN AND PHENOL RED BY THE KIDNEY[1]

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It is believed that secretion by the tubule is an important factor in the renal elimination of the dyes phenol red (1) and neutral red (2), and two organic iodine compounds, diodrast and hippuran (3). This belief rests in part on evidence that the volume of blood flowing through the kidneys is not great enough to yield a filtrate containing the amount of the substances excreted. While there is no reason to question the validity of the assumptions concerning renal blood flow in the experiments referred to, it is nevertheless true that in no single experiment has this been measured concurrently with the rate of excretion of these substances. It seemed desirable that both measurements be made simultaneously especially in the case of hippuran because, unlike the dyes, this substance may be assumed to be wholly filtrable from plasma, and hence its elimination by the glomerulus alone is not inconceivable, as appears to be the case with phenol red. The present experiments in which these simultaneous measurements have been made were devoted primarily to the study of hippuran; they were extended to include a smaller number of similar studies of phenol red. The results show that there was not enough blood flowing through the kidneys to make possible the excretion of the substances by glomerular filtration alone. Tubular secretion must therefore be a factor in their excretion by the kidney.

METHODS. The experiments were performed on rabbits anesthetized with pentobarbital or urethane. Renal blood flow was measured by a thermostromuhr (4) which was placed on the aorta. The described technique for the installation and calibration of the instrument (5) was followed except that it was placed immediately proximal to the ligated superior mesenteric artery so that blood flow to both kidneys, rather than to one, was measured. In some experiments the volume of blood flowing from the kidneys was measured without the stromuhr by means of a pipette in the vena cava according to the method of Barcroft and Brodie (6). The two methods gave similar results which will be considered together.

[1] The expenses of this investigation were defrayed in part by a grant from the Commonwealth Fund.

Hippuran[2] (sodium ortho-iodohippurate, 0.5–0.8 gram) was injected subcutaneously or intravenously from 20 to 120 minutes before clearance measurements were begun. Phenol red (0.05–0.10 gram) was given subcutaneously approximately 30 minutes before the clearance periods. In

TABLE 1

RABBIT NUMBER	LENGTH OF PERIOD	PLASMA CONCENTRATION	CLEARANCE	RENAL PLASMA FLOW	CLEARANCE / RENAL PLASMA FLOW	METHOD OF BLOOD FLOW MEASUREMENT
			Hippuran			
	minutes	*mgm. per 100 cc.*	*cc. per minute*	*cc. per minute*	*per cent*	
1	11	6.5	18.2	20.8	0.87	Stromuhr
2	10	18.5	13.0	9.9	1.31	Stromuhr
	10	12.0	9.4	8.5	1.10	Stromuhr
3	10	5.1	16.2	7.3	2.35	Stromuhr
	10	3.4	8.8	7.5	1.17	Stromuhr
4	11	4.6	9.5	15.5	0.61	Stromuhr
	11.5	6.1	6.5	12.1	0.54	Stromuhr
5	31	4.3	21.0	12.7	1.65	Stromuhr
6	12	12.2	9.5	9.6	0.99	Stromuhr
	11	10.8	2.9	7.6	0.38	Barcroft-Brodie
7	10	5.4	6.8	7.9	0.86	Barcroft-Brodie
8	15	2.8	7.9	9.4	0.84	Stromuhr
	10	3.7	11.3	8.6	1.32	Stromuhr
9	10	3.3	15.5	11.6	1.34	Barcroft-Brodie
	10	3.9	12.8	11.8	1.08	Barcroft-Brodie
Average					1.10	
			Phenol red			
6	11	8.8	2.8	7.6	0.36	Barcroft-Brodie
7	10	4.0	11.7	7.9	1.48	Barcroft-Brodie
8	14	2.3	2.8	4.9	0.58	Barcroft-Brodie
9	10	1.8	49.0	11.6	4.22	Barcroft-Brodie
	10	2.6	40.2	11.8	3.40	Barcroft-Brodie
10	10	2.7	30.5	14.3	2.14	Stromuhr
	10	3.4	44.2	14.5	3.05	Stromuhr
	10	5.0	9.7	8.2	1.17	Barcroft-Brodie and Stromuhr
Average					2.05	

some experiments either creatinine (1.0 gram), theophylline-ethylenediamine (0.012 gram) or sucrose (1.0 to 2.0 cc. of 40 per cent solution) was administered before the clearance periods to promote diuresis. Blood

[2] Hippuran was supplied by the Mallinckrodt Chemical Works.

for analysis was drawn either in the middle of the clearance period or at its beginning and end. In the latter case the samples were pooled. Urine was obtained by an in-lying catheter, care being taken to empty the bladder completely after repeatedly washing it with saline solution. Plasma and urine were analyzed for iodine by the method of Leipert (7) with slight modifications (8), and for phenol red by the method of Marshall and Vickers (9).

RESULTS. The results of 15 experiments with hippuran and of 8 with phenol red are summarized in table 1. The average hippuran clearance was 110 per cent and the average phenol red clearance 205 per cent of the volume of plasma flowing through the kidney during the clearance period. The entire elimination of these substances by glomerular filtration would require that 110 and 205 cc. of glomerular filtrate respectively be derived from 100 cc. of plasma, a manifest absurdity. It is believed that in the rabbit approximately 30 per cent of the plasma water is filtered at the glomeruli (5). If this is the case only 2 of the 23 clearances here reported can be attributed solely to glomerular filtration, in only 3 others would the filtration of 60 per cent of plasma water suffice to explain the clearances, and in 14 the filtration of all of the plasma water would be insufficient. In the latter group it must be concluded that the substances had accumulated in the kidneys in the interval between their injection and the beginning of the clearance periods. These figures stand in striking contrast to the clearance of creatinine (5), thought to constitute an indirect measure of glomerular filtrate, which requires the filtration of only 30 per cent of the plasma water. The clearance of phenol red was calculated as though this substance were present in plasma wholly in filtrable form. Only 18 per cent of the dye, on the average, was filtrable through cellophane in the present experiments. If this fact be taken to indicate an equally incomplete filtration through the glomeruli, and the appropriate correction be applied, the calculated glomerular filtrate would exceed the renal plasma flow by more than 1000 per cent.

CONCLUSIONS

Simultaneous measurement of renal blood flow and of clearance of hippuran and phenol red in the rabbit indicates that the volume of blood flowing through the kidneys in a measured period of time is insufficient to make possible the excretion of these substances by glomerular filtration alone. Tubular secretion must therefore be concerned in their elimination.

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THE EFFECT OF REMOTE LEADS UPON THE FORM OF THE RECORDED ELECTROCARDIOGRAM

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It is a well demonstrated but often neglected fact that the electrocardiogram is *a* record but not the only record which can be obtained of the action current of the beating heart. Such records are analogous and essentially comparable to the electrograms obtained from nerve and from muscle. The *details of the form* of the electrogram will depend both upon the form of the local activity in the tissue concerned and on the structure of the circuit or the medium surrounding the tissue. *The description* of this phenomenon, that is of the action current as distributed through and affected by the body fluids or other medium, might be made in various terminologies, many of which would be valid. Craib (1927, 1928, 1930) and Wilson, Macleod and Barker (1932, 1933) have interpreted the underlying phenomena in terms of dipoles. Certain aspects of this theory have been criticized by Bishop and Gilson (1929), by Gilson and Bishop (1934), by deBoer (1933), by Momm (1933), by Storti (1936) and by others. In applying a generalized terminology to the local reaction at the tissue, proponents of the "dipole theory" appear to have neglected consideration of certain essential particulars. It is our intention to point out in this paper some of the particulars which have not yet been properly dealt with under that hypothesis. We shall attempt to show that the objections which have been proposed against the generalized classical or "negativity" hypothesis are not valid and that all records which are obtained may, so far as is consistent with the data available, be rationally interpreted under the "negativity" hypothesis.

THE IRON WIRE MODEL. *a. General description of the method used.* Since the action current is a general phenomenon, the theory of its production can be illustrated on the simplest system available. We have used the iron wire model. This model offers all the essentials required, i e., activity in response to stimulation, a source of potential, a threshold, conduction of the impulse, and recovery from activity. The activity of the wire can be given some of the dimensions of that of the heart. As a simple linear structure, it is geometrically uncomplicated, and the action

currents which can be led off are comparable with those producing the electrocardiogram. The iron wire model may therefore be used to give electrograms which simulate certain features of the electrocardiogram. From such records it will be possible to check the theoretical interpretation of the latter.

If an iron wire is immersed in nitric acid of the proper strength it becomes covered with an oxide coat or *passivated*. Stimulation of the wire at a point by touching it with a strip of zinc or by passage of a local electric current causes the oxide coat in that region to break down thus *activating* the wire. The activation spreads along the wire. Its path can be followed visually by observing the liberation of gas bubbles. If studied electrically (Dériaud and Monnier, 1925; Bishop, 1927; Lillie, 1929), one finds that the surface and the adjacent acid show a change of potential; becoming more positive as the wire becomes active (wire negative). For the particular records described in this paper, we used a piece of steel piano wire (16 gauge) bent so that a 6 cm. length rested in usually 57 or 60 per cent acid contained in a circular glass dish 15 cm. in diameter.

In order to avoid drawing current from the system, arrangements were made to lead to the input of a vacuum tube (type 57) from one or both of two electrodes (as fig. 1, *T*) dipping into the acid and from a soldered connection to the part of the steel wire (*R*) projecting from the bath. For the records reproduced in figure 1, the lead-off electrodes were of passivated iron wire in some cases, of carbon in others.

Since there is a negligibly small current in the input circuit of the vacuum tube as used, and since the state of the steel wire (*R*) outside of the bath does not change during activity of the wire in the bath, the outside connection can be employed as a fixed or reference potential. One might also employ the passive surface of the wire in the bath as a zero, since it is at a constant difference of potential from the outside wire.

If now, one obtains a record during a period of activity taking one lead from the wire itself (*R* of fig. 1) and the other lead from an electrode in the bath (as *T*), there is invariably obtained a monophasic record which (with the galvanometer connections so arranged) rises above the base line, achieves a more or less level plateau, and finally returns gradually toward the base line. Figure 1C is a typical record of this sort.

b. The diphasic record as the graphical difference of the two corresponding monophasic records. The general description of the monophasic record given above, applies for any position of the electrode in the bath. Except for a possible small late "after-swing" described by Lillie (1929), it may be said that at no time in any record does the monophasic record go below the base line, that is, at no time, for any position of the bath electrode does the potential at that electrode become reversed, becoming negative with respect to the potential of resting surface. Moreover, *if the impulse is*

conducted over the entire length of the immersed wire, the height of the recorded plateau, that is, the voltage recorded during the period of

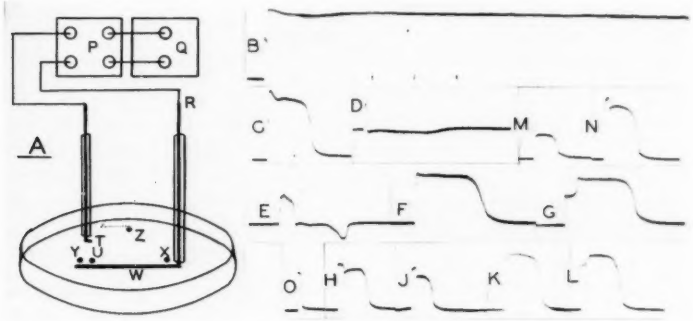

Fig. 1. A. Arrangement used in recording electrograms from steel wire. The wire (*W*), in a glass dish containing acid, was insulated above the bend by a glass tube filled with paraffine. "Monophasic records" were obtained by use of one lead from the wire at point *R* and one lead from an electrode as *T*. Diphasic records were obtained by use of two electrodes such as *T*. Electrodes were connected to the amplifier (*P*), the output of this being shunted and led to the galvanometer (*Q*).

B and C. Effect on duration of monophasic potential record of duration of activity. Leads from *Z* and *R*. For B, 50 per cent acid was used and wire had not repassivated when record discontinued at end of 11 seconds. For C, 60 per cent acid. Wire repassivated after about one second of activity.

D. Fifty per cent acid and no repassivation as for B, except that leads are from *X* and *Y*. Following the "R-wave," there is a slight fluctuation of potential but *there is no "T-wave."*

E. Leads from *X* and *Y*. Wire stimulated by touching with zinc. Diphasic record of form obtained by graphic subtraction of *G* from *F*.

F. Leads from *X* and *R*.

G. Leads from *Y* and *R*.

H. Leads from *Z* and *R*. Stimulation by zinc at proximal (bend) end. Partial conduction. Low recorded potential.

J. As for H but stimulation at distal end of wire. Impulse did not travel as far as for H.

K and L. As for H and J, respectively, but with complete conduction and full potential of activity recorded.

M. Leads from *U* and *R*. Stimulation proximal. Partial conduction. Low recorded potential.

N. As for M but stimulation at distal end. Partial conduction but nearly full potential of activity recorded because of proximity of U to active end of wire.

O. Record of brief constant current used for stimulating in B and C, of same strength but ineffective because of refractoriness of wire.

activity, does not vary with change of position of the bath electrode (*T*). However, the time of onset and the form of the rising and falling parts of the monophasic record do vary with the position of the bath electrode.

It is because these time differences exist that it is possible to record the diphasic (or polyphasic) records which are obtained when the two leads are taken from points in the bath, or, as occurs for the case of the electrocardiogram, from the surface of the body.

Figure 1, records E, F and G, illustrate a simple case of the formation of a diphasic record. Record 1E was obtained with the two leads placed in the bath and very close to the wire as at points *X* and *Y* of figure 1. Records F and G were made by the use of leads, one from the outside (position *R* of fig. 1A) and one from position *X* in the one case (record 1F) and from the outside lead (*R*) and position *Y* in the other case (1G). The wire was activated in each case by a strip of zinc touching it near the bend in the wire. It may be seen that 1F rises to a maximum more quickly and begins to recover earlier after stimulation than does 1G. This time displacement is due primarily to the time required for conduction of the impulse along the wire. Activation in this case spread relatively slowly, proceeding at a rate of about 25 cm. per second. The graphic difference of 1F and 1G, taking 1F as positive and 1G as negative gives a record of the form of 1E.

A monophasic record obtained with the bath electrode at the point *Z*, remote and symmetrical with respect to the wire, was intermediate in form between 1F and 1G but showed an equally great recorded potential (1K and 1L). The significant difference between 1F and 1G is therefore not the height but rather the time at which the maximum potential (or plateau) is reached, and the time when return from that potential begins. The time at which the crest is attained is a function of the position of the electrodes and of the time elapsing during the conduction of the impulse. It is therefore misleading to assume, as the proponents of the "dipole theory" have done, that an electrode placed in the bath, at a point remote from the active tissue, remains at fixed potential before, during and after activation of the tissue. It does not remain fixed either with reference to the potentials of the poles of the dipole, or with reference to a potential half way between them.

It has been stated above that during the period of activity of the wire, the monophasic record shows a sustained potential which is essentially the same at whatever position the bath electrode be placed. Record 1B was recorded from a wire in acid so diluted (50 per cent) that the wire, once activated failed to repassivate. The action potential was still maintained when the camera was stopped after a period of activity of about eleven seconds. The maintained action potential is evident from the record. It is obvious that with respect to either the wire at point *R*, or to the potential of the previously passive surface, the whole bath including the distal electrode has changed potential.

c. The monophasic potential with partial conduction. By alternately

stimulating first one and then the other end of the wire at proper intervals, a situation arises such that conduction spreads only part way along the wire. When the bath lead is placed remotely and symmetrically with respect to the wire, as at point *Z*, it makes no difference in the form or in the height of the record whether stimulation takes place at the proximal end of the wire (near the bend) or at the distal end. If conduction proceeds only about half way along the wire, the recorded monophasic potential, which is the potential attained at *Z*, is about half that obtained when there is conduction the full length of the wire. Under such conditions of partial conduction, with leads from *R* and *Z*, records such as 1H and 1J are obtained. These may be compared with records 1K and 1L taken by the same leads but with conduction proceeding the entire length of the wire. Supplementing the above, records such as figure 1M and 1N leave little room for questioning the interpretation of the records.

If conduction is complete, the amplitude of the recorded potential difference during activity is the same between *R* and *X* as it is between *R* and *Z*. On the other hand, since the potential difference between *R* and *X* is nearly or quite the same whether conduction proceeds the full length of the wire or only part way from the proximal end, a potential difference must exist between *X* and *Z* under the condition of partial conduction. In brief, the potential attained at *Z* when conduction has proceeded half way along the wire from *X* has a value approximately half way between the potential at *X* and the potential at *Y*. At this moment there exists a condition more or less equivalent to the dipole of Craib and of Wilson and his collaborators (see also figs. 2A, 2B, and 4). However the potential at *Z* at this very particular moment is neither a desirable nor a proper potential of reference. The potential at *Z* changes in relation to the potentials either at *X* or at *Y* or at *R* according to the fraction of the area of the wire which is active, even though the actual difference of potential between any active point and any inactive point on the wire may be a constant.

The material treated in this section illustrates two related difficulties in the argument of Wilson, Macleod and Barker. These are first, the practical complication of assuming that a remote point is suitable to be tak as a fixed zero potential even though it is the mid-value of a hypothetical dipole having constant potential difference, and second, the failure to take into account the fundamental theoretical effect of change of the relative areas of the parts active and inactive, respectively, that is, of the effective potential source and sink, on the distant electrode. The following section deals with the general case, i.e., the effect of such areal relationships as they can be demonstrated on a static model.

DISTRIBUTION OF POTENTIAL ABOUT CHARGED WIRES IN A PLANE CONDUCTOR. Figure 2A is a plot of the theoretical distribution of isopotential

lines in an infinite plane conductor about the dipole AB. For this distribution, the poles A and B must be considered as negligibly small in size and of equal and opposite sign. The contour lines indicate levels of isopotentiality so that there is no potential difference and therefore no flow of current between any two points on any given contour line. The isopotential lines in this case constitute two series of co-axial circles.

Figure 2B is a plot of isopotential lines, as measured on a salt-soaked filter paper, for the field about two wire electrodes of equal and finite size (3 cm. length). The contour lines are considerably distorted from the circular form but the field is symmetrical about the line perpendicular to and cutting the axis at the middle of the dipole, and this midline perpendicular is at a potential midway between the potentials applied to the two poles respectively. This case is a rough representation of the condition holding when the impulse has been propagated half way along the iron wire (or heart strip or heart).

In contrast to figure 2B, figure 2C is a plot of several isopotential lines about electrodes with lengths of 5 cm. and 1 cm., respectively. The field about these electrodes is grossly distorted and the potential half way between the potentials applied to the electrodes lies on a contour line which would pass through or near the point P. Such a model would represent either a moment early or a moment late in the spread of activity along the preparation.

The plots described above were made only over 20 cm. of a 40 cm. saline-soaked filter paper. A severe distortion of the fields becomes apparent when one approaches close to the boundary of the plane or solid conductor considered. This additional distorting factor applies, of course, when leads are placed on the surface of the body.

When the iron wire is activated, its surface changes from a condition diagrammed as figure 2D to a condition diagrammed as figure 2H, intermediate stages being as 2E, 2F and 2G. The preparation thus changes from the resting condition (2D) where the entire surface is at the resting potential and when therefore the entire bath is at the potential of resting surface, to a condition (as 2H) where the entire surface is active and at the potential of activity and when therefore any point in the bath is at the potential of activity. During the recovery process, a reverse process takes place. In the case of the beating heart, unequal distribution of areas along a circuitous path of impulse distribution would, of course, add a further complication to the analysis of the distribution of potentials at points in a surrounding bath.

It should therefore be apparent that a complete knowledge of the local changes occurring at the surfaces of the preparation might lead to a correct prediction of the potential difference arising between the lead-off electrodes placed in the bath. On the other hand, knowing only the latter,

it would be difficult or impossible to predict the former. The latter problem is precisely that involved in the interpretation of the electrocardiogram. In the following section there will be considered the extent to which such interpretation, based on physical principles, can be carried.

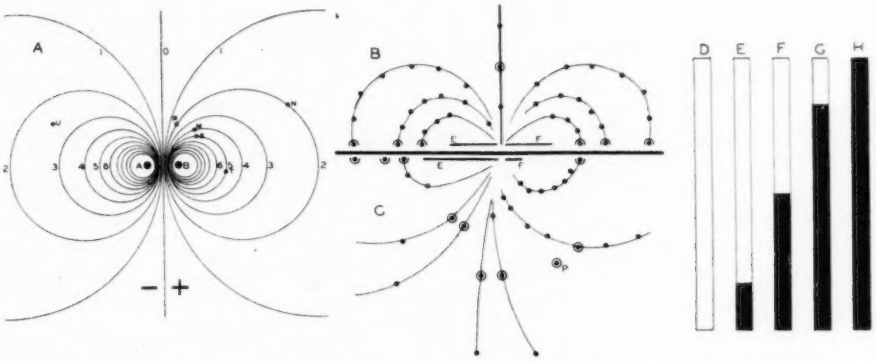

Fig. 2. A. Theoretical distribution of isopotential lines around the dipole AB in an infinite plane conductor. A and B are considered as of negligibly small size and to be equally and oppositely charged.

B. Plot of several isopotential lines about two 3 cm. electrodes (*E* and *F*) placed on wet paper. Only the upper part of the field plot is reproduced. The lines are distorted from the circular form seen in 2A but the field is symmetrical.

C. Similar plot made by using electrodes 5 cm. and 1 cm. long respectively (lower half only reproduced). Due to the unequal area of the electrodes, there is a severe asymmetry of the current lines and consequently of the potential lines drawn. Whereas in 2B the potential midway between that of the potentials applied to the electrodes lies along the line perpendicular to and passing midway between the electrodes, in 2C this mid-potential lies on or near a contour line which would pass through point *P*.

In terms of figures 2B and 2C, the changes in the potentials of the field surrounding the poles in a conducting medium, when one pole increases in area and the other decreases, amount to a progressive shift of the diagram as represented in 2C through the symmetrical condition of 2B to the mirror image of the first condition (2C).

D to H. Diagrams indicating the change of the tissue surface from one of total resting surface (2D) when the entire bath is at the potential of resting surface, to the condition of complete activity (2H), at which time all points in the bath are at the potential of resting surface. E, F, and G represent intermediate stages giving field conditions comparable to those of 2B and 2C.

In relation to the foregoing, and in anticipation of what follows, a clear statement is in order as to the significance of a zero of reference with which to compare the potential at a given locus. It will be obvious at once that if *any* point is fixed, say by connecting it to earth, other points will change with respect to it. The choice of the point to be taken as a zero of reference is a purely arbitrary one, determined by considerations of convenience and not by logical necessity. The recorded datum is not

the potential *at* a point but the *difference* of potential *between* points. To say that the presence of a dipole does not change the potential at a distant point (*vide* Wilson, Macleod and Barker) simply means that the difference of potential between the distant point and one pole is equal and opposite in sign to that between the point and the other pole. This applies only for special cases, and fails for instance, when a region of positivity partially or completely surrounds a region of negativity as is the case, in general, for tissues according to the membrane theory. Such a statement certainly fails under any theory for the iron wire model except at the particular instant when one half the wire is active. The same must hold for tissues. To infer, therefore, that a given region (ahead of the excitation front) becomes positive to a distant point *which has not changed in potential* seems to us to be misleading in its implications, and the argument attempting to justify such a statement is neither right nor wrong, but merely beside the point when applied to active tissues. In particular, such a "positivity" does not indicate that the positive pole of an active plus-minus couple is located at the region of apparent positivity, or even directed toward it. This positivity might equally well be assigned to a passive *ir* potential gradient due to a potential source elsewhere. Such passive potential gradients must certainly extend to some distance from the site of "activity" in tissues surrounded by conductors. However, to surround a tissue by a fluid conductor in such studies is to make use of an arrangement peculiarly unsuitable for analyzing tissue activity.

DISTRIBUTION OF POTENTIAL IN THE CONDUCTING MEDIUM SURROUNDING THE BEATING HEART. Despite differences in structure, in composition, and in the direction of the external potential change during activity, the systems discussed above show a distribution of potential in a surrounding medium which is strictly analogous to that surrounding the beating heart *in situ*. Figure 3 presents records obtained from a turtle heart *in situ*. For these records, the brain of the turtle was destroyed, the plastron removed, the pericardium opened widely, and ball-tipped silver electrodes used for leading to the amplifier which activated the oscillograph galvanometer. The exposed (ventral) part of the ventricle was kept wet but was slightly above the level of the surrounding pericardial fluid. We shall consider here only the details of the initial ventricular complexes (Q-, R-, and S-waves).

The records of figure 3A, 3B, 3C and 3D were obtained with the ground electrode (so connected as to cause an upswing of the record when it became relatively negative) on the ventricle near the apex. The grid electrode was moved from one point to another near the outer margin of the body cavity for the different records. The records have nearly the same form in all the cases.

In contrast to the first group of records of figure 3, figures 3E, 3F, 3G

and 3H show records obtained by keeping the grid electrode fixed in the pelvic region of the body cavity, whereas the ground electrode was moved from a position near the A-V funnel (3E) to the lateral basal margin (3F), thence to a point near the apex (3G) and finally to the extreme apex of the heart (3H). It is to be seen that in this latter group of records there is a progressive increase of the downswing of the record as the ground electrode is placed nearer and nearer to the apex, that is nearer and nearer to the last point of the ventricle to be activated.

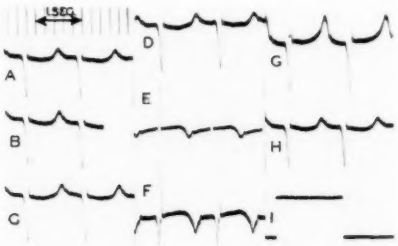

Fig. 3. Records made from the ventricle of a spontaneously beating turtle heart. Galvanometer so connected that relative negativity of the ground electrode resulted in an upswing of the record, relative negativity of the grid electrode resulted in a downswing of the record. For figures 3A to 3D inclusive, the ground electrode was kept fixed near the apex of the ventricle. The grid lead was placed for 3A in the pelvic region of the body cavity, for 3B at the base of the neck, for 3C at the base of the right foreleg, for 3D at the base of the left foreleg. The records have a great similarity of form.

For figures 3E to 3H inclusive, records were made with the grid lead fixed in the pelvic region of the body cavity. The ground lead was placed for 3E close to the A-V groove on the left side of the heart, for 3F on the left basal lateral margin, for 3G part way down the ventral surface of the ventricle, and for 3H on the ventricular apex. 3I indicates the adequacy of the amplifier used for recording slow changes in potential. The excursion is in response to a 10.5 mv. input to the amplifier used at the same sensitivity as that for the above records.

Taking 3F as an example and considering the heart to be surrounded by an inert conductor, the interpretation of any of these records is as follows. With the beginning of ventricular activation, the remote (grid) electrode is subjected to a relative negativity which results in a downward swing of the record, since an electrode so placed is at a point at which there is a potential approximately an average of the potentials at the various points of the heart surface. The contact (ground) electrode, relatively insulated and in contact with resting heart surface, remains at a potential close to that of resting heart surface. As the impulse advances toward and reaches the surface under the contact electrode, there is the sharp upswing of the record. If the impulse has not spread completely over the tissue,

the average potential of its surface is not yet at the full potential of active tissue and the potential at the remote electrode recording this is not yet maximum. The contact electrode, however, achieves the full potential of active tissue and the swing extends above the base line as the R-wave. When the entire surface has become activated, the record has returned to the base line because the two electrodes are now at the same potential but not because there has been a return of the potential of the distal electrode to the potential of resting surface. When the contact electrode is properly placed at the apex, there is no swing above the base line, the contact and remote electrodes coming to the same potential at the same time which is the time at which the last point on the ventricle becomes active. (The spread of the excitation wave over the surface of the turtle heart does not follow the course which apparently holds for the mammalian heart.) Thus, despite the fact that *relative electrode negativity* at the contact electrode causes an upswing of the record, 3D shows (or might show) only a downward excursion. In other words, contrary to the interpretation of Craib and of Wilson and his collaborators that a remote electrode remains at fixed potential, we have here a case in which the galvanometer deflection depends predominantly upon the development of a difference of potential between such a remote electrode and the still inactive surface of the heart.

The relative constancy of form of the first records of figure 3 is therefore not due to the fact that there is no change of potential at a remote electrode but rather because there are only slight differences in the potentials developed at any given time *between any two remote points.*

For the heart strip in air, the forms of the records obtained (with leads not at the ends of the preparation) are in general accord with those which might be predicted under the "dipole hypothesis." Such a preparation, because it is in an insulating medium (air), thus approaches the electrostatic condition from which the theory was derived. In such a preparation during the period of activation or during the period of recovery, one can consider that there exist 1, a constant potential difference between a dipole interface, the active-inactive interface, and one end of the tissue, and 2, a directionally different but constant potential from this interface to the other end of the tissue. This statement can be made without one's becoming involved in the question of how the two areas in question came to be different, how they affect a remote lead, or how they vary during the propagation of or recovery from activity. This is because the strip is in an insulating medium so that no current flows across the potential boundary external to the strip.

The difference of potential across the active-inactive border of the strip is constant because there is no flow of external current and there is a constant internal current. On the other hand, when the preparation is

immersed so that there is shunting due to the immersion fluid, the distribution of the flow of current in the external circuit reflects the relative areas of active and inactive tissues involved (see figs. 2B and 2C). This distribution of current flow determines, as an *ir* drop with respect to the areas of active and inactive surface, the potential of any point in the medium. The records obtained with two remote leads are therefore records of the *difference of potential* between different points in the bath but are not true records of local changes occurring at the heart surface.

Discussion. During the spread of the wave of activation over the surface of the immersed heart (or heart strip or iron wire), the potential at a relatively distant point changes from the full potential of the resting surface to the full potential of the active surface. A more or less sym-

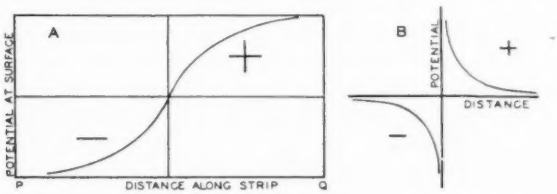

Fig. 4. A. Diagram of the approximate distribution of potential along the surface of a wet or immersed strip of tissue when the impulse has been propagated half way along the strip.

B. Diagram such as that used by Craib and by Wilson, Macleod and Barker to indicate the distribution of potential with respect to an hypothetical dipole such as that of figure 2A. Distribution such as this could not be found in fact as a general case at the surface of a tissue although a record having a similar form (as the initial complex of fig. 3F) can be obtained when one lead is placed near the mid-point of excitation spread and in contact with the tissue and the other lead is placed in the conducting medium sufficiently removed from the tissue to give an averaged potential from its entire surface.

metrical record such as 3F lead with one contact electrode and one remote electrode represents a special case in which the contact electrode is at a point activated at a time near the middle of the total time of activation for the chamber of the heart concerned. At such a time, if the tissue be diagrammed as in figure 2F, the distribution of potential along its surface would be something as diagrammed in figure 4A. A simple diagram of potential distribution (fig. 4B) such as that used by Craib (1930, p. 14, fig. 3) and by Wilson, Macleod and Barker (1932, p. 432, fig. 4) would be applicable to the immersed tissue preparation only if 1, the assumption of the simple, constant dipole were valid for representing tissue activation, and 2, if the exploring electrode were moved, relative to the dipole, in a constant direction and along the axial line through the dipole. Such an axis would lie *within* the preparation and points on the tissue surface would

not lie upon the axis but would correspond to remote points as *M*, *N*, etc., of figure 2A.

Since 1, the potential at a remote point is not valid as a fixed reference potential, and since 2, the changing, during the period of activation, of the relative areas active and inactive, results in a changing of the constants of the effective or equivalent dipole from moment to moment, the so-called "dipole hypothesis" as developed by its proponents cannot be regarded as a valid description of the changes occurring *at the surface of the tissue*. The failure to consider the facts referred to at the beginning of this paragraph has resulted in considerable confusion in recent literature.

The dipole theory in the form elaborated by Wilson, Macleod and Barker is based upon an analogy between the electrostatic fields about a pair of charged particles, and the electrodynamic fields due to flow of current and consequent *ir* drop of potential in a conducting medium about two sources of current (source and sink). This analogy holds for the simple case as stated by the above authors, when the poles can be considered as points close together, resulting in a field distribution symmetrical about a perpendicular to the axial line through them. It might be made to apply even when unequal and changing areas of potential source during the rise and decline of activity compromise the simplicity or even the correctness of the deductions which made the theory so attractive. Since the "dipole theory" as so modified reduces to the "negativity theory," it is problematical whether the dipole theory thus complicated by the necessary considerations, would be a material improvement on the classical membrane theory. Without these considerations which have so far been neglected in the development of the "dipole theory," the theory is not even adequate as an alternative.

With respect to the surface of the heart itself, the surrounding fluid, in a valid sense, acts merely as an extension of the remote electrode as we have previously stated in the paper to which Wilson, Macleod and Barker take exception. If one is interested in knowing the changes occurring at the heart surface, he will best consider the potentials as recorded from points as near the heart surface as possible.[1] Interpretation is perhaps most

[1] Wilson, Macleod and Barker (1933, p. 11) object to the following and similar statements, "The bath as a whole, acting as an electrode lead, will become relatively negative to the inactive region of muscle" (Bishop and Gilson, 1929, p. 141). We believe that this statement is correct within its context, but could, perhaps, have been put more precisely. For instance, a distant lead in the medium will be at a potential which is an average of the potentials of all points on the tissue surface with which the medium is in contact, while a proximate lead will be at the potential of the surface adjacent to it. During spreading activity, therefore, the average potential of the surface as recorded by a distant electrode will be somewhat negative to the inactive surface as recorded by an electrode in contact with it, but not as negative as is the active surface. In this sense, the medium itself acts as a diffuse electrode,

easily made from the moist strip preparation in air. Such a preparation is one in which there is at least an approach to propagation along a linear and uninjured tissue. As is well appreciated by any who have worked with this preparation, the approach is at best remote.

When the heart is immersed in fluid and the electrodes are removed farther and farther from the heart, a valid interpretation of records includes a much more limited scope. For determining moments of activation of various points over the heart surface, the method of one contact and one remote electrode may be valid for the moist preparation, the moment of the sharp upswing of the record corresponding to the moment of activation under the contact electrode. If the contact electrode is exposed to the surrounding fluid, its value as a localizing electrode becomes compromised.

In the case of the clinical electrocardiogram, the leads are effectively from the surface of the immersing bath. By use of various leads and of a considerable mass of accumulating empirical data, certain rather gross localizations may be deduced. However, as Craib has pointed out, even a limited analysis of local changes can be made directly from the record only under favorable circumstances and with a highly circumspect interpretation.

SUMMARY AND CONCLUSIONS

1. The material which has been presented deals with the distribution of potentials in a conducting medium surrounding hearts or inorganic models.

2. It has been shown that the so-called dipole theory of tissue potentials as expounded by its proponents is not adequate because:

a. The nature of the spread and duration of the activity in a tissue such as the heart, in which the duration of the sustained activity is greater than the time required for the spread of the impulse through the chamber under consideration, are such as to preclude the assumption of a moving, constant dipole.

b. When the entire heart surface has changed from a condition of rest to a condition of activity, any change of potential involved must be accompanied by a change in the potential of the whole medium with which the heart is in contact, since no difference of potential exists, either during rest or during the completely active state, between this surface and any point in the medium. The assumption that a distant point in this

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the distant electrode in it measuring the mean potential of its whole contact with the tissue, as contrasted with a near electrode measuring the potential at a localized region. In this form the statement is merely an application of the statement by Wilson, Macleod and Barker concerning the effect of a dipole on a distant electrode, extended to a surface with a non-uniform charge, such as is actually found in the case of tissues.

medium does not change in potential with change from passive to active state of the heart is therefore misleading and physiologically is beside the point, since such an assumption is derived from the erroneous notion that the active process in heart tissue can be adequately treated as a simple dipole moving in space.

3. A lead in contact with the moist surface of the heart in air tends to record with considerable localization the potential changes occurring at the heart surface immediately beneath it. On the other hand, any electrode in the bath and removed from the heart surface tends to record an averaged potential from the entire heart surface. The actual record obtained with leads in whatever position is, of course, a record of the potential *differences between the two electrodes.*

4. It is urged that, although expediency often demands, as for the case of the clinical electrocardiogram, the use of remote leads, nevertheless, records obtained by use of such leads should not be considered as offering valid *a priori* or complete evidence concerning the nature of the local potential changes at the heart surface. It should be obvious that despite this, the electrocardiogram offers a valuable aid in studies of the heart but it has this value only because of the accumulation of empirical clinical data and the correlation of this material with data obtained by direct experimentation.

5. It is not considered that the arguments offered in support of the so-called dipole theory are such as to warrant abandonment of the classical or so-called negativity hypothesis as fundamental in the analysis and interpretation of electrograms from active tissues.

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A STUDY OF THE RELATION OF THE SPLEEN TO ERYTHROPOIESIS AND RED CELL DESTRUCTION IN THE GUINEA PIG

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I. ERYTHROPOIESIS. The influence of the spleen on red cell formation has already been a subject of extensive investigation. Although the normal adult mammalian spleen is no longer an erythropoietic locus, attempts have been made to establish an indirect relation of this organ to red cell production. Thus the researches of Pearce and Krumbhaar (1918), among others, have shown that shortly following extirpation of the spleen from dogs, there develops a mild or moderate secondary anemia, which attains its most intense stage in four to six weeks. These experiments have suggested that, with the removal of the spleen, the animal has been deprived of a substance, possibly hormonal in nature, which is necessary for normal erythropoiesis. In direct opposition to this view, either because of an increase in red cell count or of the presence of Howell-Jolly bodies sometimes observed after splenectomy, Stradomsky (1917) and others have claimed that the spleen exercises an inhibitory effect on the bone marrow.

If the spleen influences marrow activity, not only should the red cell count change after splenectomy, but the application of a powerful erythropoietic stimulus would be expected to elicit a different response in splenectomized animals from that in normal animals. The first part of this paper is concerned with such a study.

MATERIALS AND METHODS. 1. *Animals.* The young adult guinea pig (weight 350 to 400 grams), was chosen as the experimental animal for two reasons: 1. The guinea pig is not prone to infection after removal of the spleen as are other animals, particularly the rat and dog, which sometimes succumb to Bartonella diseases (Perla and Marmorston, 1935). 2. It is well known that in young animals the spleen is more active and the ratio of spleen to body weight is greater than in older ones. Measurements have shown the weight of fresh spleen in the 350 to 400 gram guinea pig to range from 0.15 per cent to as high as 0.3 per cent of the body weight, which is quite large compared to that of mammals such as the rabbit or cat, and of the same order of magnitude as that found in monkey and man (Krumbhaar, 1926).

2. *The erythropoietic stimulus.* The stimulus employed was a low oxygen tension, obtained by placing the animals, for ten days, in a low pressure chamber, through which fresh air was constantly drawn (for details as to the construction of the chamber, see Dubin, 1934). We have used a pressure of 370 to 380 mm. Hg, which is sufficiently low to cause the outpouring of a significant quantity of red cells into the circulation of the normal animal. The animals were removed from the tank once a day for about 30 minutes to clean the cages and to supply food, which consisted of fresh greens and Purina chow.

3. *Counts.* Red blood counts were made from freely flowing samples of blood drawn from the ear,[1] each of us counting 400 squares on a Levy-Hausser hemacytometer chamber. If we did not agree to within 2.5 per cent, recounts were made until they fell consistently within this limit of error.

Reticulocytes, expressed as a percentage of the total reds, were counted in wet mounts vitally stained with brilliant-cresyl-blue.

Results. 1. We have found that, although a moderate post-splenectomy anemia occurs, this anemia is not peculiar to the removal of the spleen but takes place upon the extirpation of other organs, or even when mere abdominal incision is made under anesthesia. This can be seen from table 1, where the counts of five representative splenectomized animals are compared with those of five representative animals subjected to other surgical operations. The numbers of red cells are given in millions per cubic millimeter. The reticulocytes are expressed as a percentage of the total red cells.

Inspection of the table will show that the extent, the course of the anemia, and the time for return of the red cell count to normal are approximately the same in all the animals. This anemia is not due to loss of blood. There is very little hemorrhage attending the removal of the spleen or the other organs, and still less in simple abdominal incision. This is substantiated by the behavior of the reticulocyte count. Moderate hemorrhage results in a significant increase of reticulocytes, but this was found in only one animal in the entire series (no. 1), and even here the drop in red cells was not as great as in other animals which showed no significant reticulocyte rise.

We have done experiments which eliminate the possibility that the anesthetic employed (ether) produces the anemia. Animals subjected to ether anesthesia for three 30 minute intervals over a period of four weeks showed no significant drop in red cell count. The remaining possibility is that the anemia is produced by the incision, due perhaps to the release of substances from the cut tissues. The removal of organs does not intensify the anemia, as shown in table 1.

[1] We have frequently checked counts on blood taken from the ear against samples drawn from the heart and have found no significant difference between the two.

2. Another way of determining whether the spleen is related to erythropoiesis would be to compare the response of splenectomized and control animals to the low pressure stimulus. Consequently a group of 69 splenectomized and 41 operated control animals kept from 3 to 78 days after the operation before being subjected to the stimulus, were exposed to the reduced pressures for a period of ten days. The results show that the stimulus produces an increase in the red cell count ranging from one to two millions per cubic millimeter, and a reticulocyte count of .8 per cent to 10 per cent in all the animals regardless of their treatment. Thus it demonstrates that the increased activity of the erythropoietic system in the guinea pig, in response to an erythropoietic stimulus, is not dependent on the presence of the spleen.

TABLE 1

NUMBER	OPERATION	COUNTS BEFORE OPERATION		COUNTS AFTER OPERATION											
				One week		Two weeks		Four weeks		Six weeks		Eight weeks		Ten weeks	
		Reds	Reticulocytes	Reds	Reticulocytes	Reds	Reticulocytes	Reds	Reticulocytes	Reds	Reticulocytes	Reds	Reticulocytes	Reds	Reticulocytes
1	Splenectomy	6.1	0.2	5.9	0.8	5.5	2.0	6.0	0.2	6.0	0.2	5.9	0.3		
2	Splenectomy	6.1	0.4	5.4	0.6	5.2	0.3	6.0	0.1			5.9	0.1	5.9	0.1
3	Splenectomy	6.0	0.2	5.2	0.7	5.1	0.2	6.0	0.8	5.8	0.7			5.8	0.8
4	Splenectomy	6.3	0.3			5.8	0.4	5.2	0.3	5.2	0.3			6.1	0.2
5	Splenectomy	5.7	0.4	4.9	0.3			5.0	0.3	5.0	0.2			5.4	0.6
6	Laparotomy	5.8	0.7			4.8	0.4	6.1	0.7						
7	Unilateral nephrectomy	5.8	0.1	4.4	0.4	5.2	0.4	5.4	0.1			5.4	0.1	5.6	0.2
8	Laparotomy	6.1	0.1	5.3	0.4	5.2	0.3	5.4	0.6	5.4	0.9	5.8	0.2	6.0	0.1
9	Unilateral gonadectomy	5.9	0.1	5.4	0.8			5.1	0.1	5.0	0.1	5.3	0.3	5.8	0.4
10	Laparotomy	6.1	0.1	5.3	0.1			5.2	0.4	5.5	0.1			5.7	0.4

II. Red cell destruction. Ponfick and Kölliker (see Krumbhaar, 1926) were among the first to bring attention to the spleen as an organ of blood destruction, and described the erythrophagocytic activity of the reticulo-endothelial elements. Since that time, the rôle of the spleen in destroying red blood cells has been well established by numerous investigators, particularly Pearce and Krumbhaar (1918), and Asher (1935). In addition, a more obscure series of investigations has pointed to the possibility that the spleen is concerned with the elaboration of some substance which weakens or prepares the red cells for destruction. This is essentially the basis of Botazzi's (1894) hemocatatonistic and Banti's (1913) hemolytic theories.

Although *in vitro* changes in resistance of red blood cells to certain hemolytic agents following removal of the spleen have been reported, no

experiments have as yet been performed which conclusively demonstrate that these changes also occur within the organism. Since we have at our disposal a method of ascertaining the rate of red blood cell destruction within the animal, we have thought it advisable to relate these *in vitro* resistance changes to any changes in the *in vivo* rate of red cell destruction which might occur following splenectomy.

METHOD. 1. As we shall show, red blood cell destruction within the organism may be studied by stimulating the erythropoietic centers and then following the behavior of the red cells and reticulocyte counts during their return to normal levels. In order to produce an increase in the numbers of circulating red blood cells, splenectomized and normal guinea pigs (350–400 grams) were subjected to low pressures of 370 to 380 mm. Hg for ten days.

2. *Hemolysis tests.* The resistance of red cells from blood taken by cardiac puncture from splenectomized and control unilaterally gonadectomized, unilaterally nephrectomized, laparotomized and partially omentectomized animals was tested with saponin, sodium taurocholate, and hypotonic saline, in a water bath thermostatically controlled at 30°, at various periods following the operation. For determining changes in resistance to saponin and sodium taurocholate, essentially the same method as described by Ponder and Yeager (1930) was employed. Briefly, this consists in noting the times for complete hemolysis of red blood cells suspended in 1 per cent NaCl, produced by different concentrations of the lytic agents. The solutions of lysin were freshly prepared before use, and the red cell suspensions were always of the same concentration.

Fragility tests in hypotonic saline were conducted as follows. Packed cells of oxalated blood were washed three times and suspended in 1 per cent NaCl. A fixed quantity of this suspension was added to dilutions of the NaCl, so prepared that the resultant total volumes would be equal at the different tonicities. In those tubes where lysis was delayed, the systems were allowed to stand in the water bath for 90 minutes, and at the end of this time the greatest concentration of NaCl which produced complete hemolysis was recorded.

RESULTS AND DISCUSSION. A. *Red blood cell destruction in vivo.* The behavior of the red cell and reticulocyte counts following cessation of the low pressure stimulus can be summarized as follows: 1. In all the 34 unoperated and operated (placed in the chamber varying periods of time after the operation) control animals, the red cell count did not rise above the figure attained at the cessation of the stimulus and began to fall upon its termination. 2. In the splenectomized animals, however, the behavior of the red cell count depends on the length of period elapsing between the operation and the application of the stimulus. In these animals, the red cell counts continue to rise above the count obtained on removal from the

chamber, maximum rises occurring in animals subjected to the low pressures 10 to 20 days after splenectomy.[2] The intensity of these post-exposure rises begins to diminish in animals placed in the low pressure chamber after a period longer than 20 days subsequent to splenectomy, animals kept two and a half months before being exposed to the stimulus finally showing no rise above the exposure level, as in the controls. The results thus indicate that the rise above the count obtained upon removal from the chamber is greatest approximately 25 to 35 days after splenectomy.

There are several possible explanations for the occurrence of these post-exposure rises. 1. In these animals, the marrow may continue to operate at a high level even though the stimulus has been withdrawn. This is not the case since the reticulocyte counts, an index of marrow activity, fall at the same rate and reach normal levels within 4 to 6 days in all the animals. 2. Another explanation is that they are due to a decrease in plasma volume but, if this were the case, it is difficult to see why the post-exposure rises do not become maximal immediately, but only 25 to 35 days after removal of the spleen. 3. A third possibility is that with the removal of the spleen, a large portion of the reticulo-endothelial system, normally concerned with red cell phagocytosis and destruction, has been excised but, again, if this were the only factor concerned, it is difficult to explain why the post-exposure rises are not as great immediately after removal of the spleen as later on. 4. Finally, it may be true that in animals deprived of their spleens, some factor is operating which results in an increased resistance of the red blood corpuscles. If, because of the presence of the spleen, the cells are less resistant it would follow that, after splenectomy, the resistance of the cells would not immediately be at maximum, but would attain a maximum value when all the cells which had been subjected to the action of the spleen have been removed.

Using the method of Escobar and Baldwin (1934), it may be calculated from our data that the longevity of the erythrocyte in the splenectomized guinea pig is approximately 30 days. Thus the period 30 days after splenectomy corresponds to the time taken for the cells previously acted upon by the spleen to have been replaced by the more resistant cells coming from the marrow. As can be seen, this period coincides with the time following splenectomy when the post-exposure rises were greatest.

B. *Hemolysis experiments in vitro.* 1. *Resistance to sodium taurocholate.* Since we know the dilutions of lysin and the times for complete hemolysis we may plot the time-dilution curves describing the destruction of red

[2] That these rises are not affected by a post-operative anemia can be seen from our data which show that the height of the rise of the red cell count due to the stimulus, and the rate of return, are approximately the same for all the operated and unoperated controls, regardless of the time after operation they are subjected to the low pressures.

cells by sodium taurocholate for animals at varying periods of time after splenectomy, and those for the same animals before the operation. These curves show that, after splenectomy, the resistance of the red blood cells to 5 dilutions of taurocholate increases, reaching a maximum approximately six weeks after the operation and then decreases, being still slightly above normal values after 5 to 6 months. The resistance of the red blood cells of operated controls remained unaltered for longer than 6 months following the operation.

2. *Resistance to hypotonic saline.* As with taurocholate, an increased resistance of the red cells to complete hemolysis by hypotonic saline was observed. The resistance reaches a maximum approximately 6 weeks after splenectomy, the greatest concentration which produces hemolysis at this time ranging from 0.25 per cent to 0.275 per cent NaCl against 0.35 per cent to 0.40 per cent NaCl for the controls. The fragility also approaches normal limits after 5 to 6 months. Red cells from the oxalated bloods of operated control animals exhibited no such changes in resistance.

3. *Resistance to saponin.* In experiments conducted on the same samples of blood and under the same experimental conditions as for the resistance tests to taurocholate and hypotonic saline, no differences in resistance to saponin were detected in any of the splenectomized and control animals.

The fact that the resistance of the red cells changes with respect to some types of lysis and not to others argues for different sites or methods of action of these agents. Whatever the case may be, it appears that the spleen acts upon the red cell membrane so as to affect taurocholate and hypotonic saline lysis, but not that produced by saponin.

Figure 1 shows how the *in vitro* changes in resistance to sodium taurocholate are related to the rate of red cell destruction within the organism, following splenectomy. In indicating the changes in resistance to taurocholate, the values of R_∞ are employed. These values, derived from the time-dilution curves, represent the ratio of the asymptotic concentrations of lysin which will produce complete lysis of the red cells of the splenectomized animals to the asymptotic concentrations of lysin necessary to cause complete hemolysis of the cells of the same animals before the operation. A value of R_∞ greater than 1.00 is thus a criterion of an increased resistance. (For a discussion of the theoretical derivation of R, the reader is referred to Ponder and Yeager, 1930.)

Two explanations may be offered for the fact that the rate of red cell destruction within the organism following splenectomy becomes normal months before the red cell resistance to certain lytic agents returns to normal values: 1, that as a result of the removal of the spleen, accompanying the increase in resistance of the cells, an hypertrophy of the reticulo-endothelial system in other organs has occurred, and 2, that each phagocytic element in the remainder of the reticulo-endothelial system has

become more active. The first explanation is supported by facts quite commonly stated in the literature. Goebel and Miller (1935) have recently made a quantitative study of the increase in the number of Kupffer cells in the livers of dogs following splenectomy. The second explanation receives corroboration from our own observations which have shown that although no signs of red cell phagocytosis can be detected in the livers of normal guinea pigs, after extirpation of the spleen many of the liver reticulo-endothelial cells can be seen swollen with red cell fragments and hemosiderin particles. Thus it appears that an equilibrium is reached in the organism where, although the resistance of the red cells is still above normal for a relatively long period after splenectomy, the compensatory

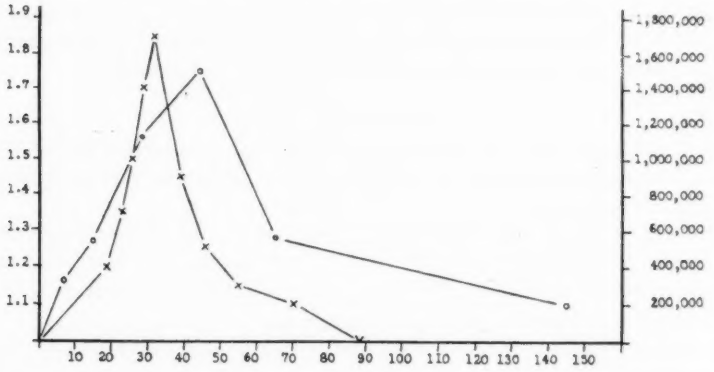

Fig. 1. Abscissa—days after splenectomy; left ordinate—R_∞; changes in values of R_∞ are shown by graph drawn through circles; right ordinate—rise of red cell count above that attained on termination of stimulus; changes in these values are shown by graph drawn through crosses. Each cross or circle represents the data of groups of at least 6 animals which showed little variation.

hypertrophy and increased activity of the remaining reticulo-endothelial system elements occurs to such an extent that destruction of the red cells in the splenectomized animals soon takes place at the same rate as in the controls.

Several investigators have attributed this increased resistance of the red cells to an increase in the cholesterol content of the blood after splenectomy. This rise in cholesterol was not corroborated by Dubin and Pearce (1916) in dogs. Moreover, if an increase in cholesterol is really the factor causing the increased resistance, it seems likely that the resistance of the red cells would not take 6 weeks to attain a maximum, but would be high soon after the removal of the spleen. Finally, we have done experiments which show that the subjection of red cells to as much as 15 times the

concentration of cholesterol normally present in guinea pig blood does not alter their resistance to taurocholate or to hypotonic saline.

Orahovats (1926) explains his finding of a decreased resistance to hypotonic saline hemolysis of the cells of splenic pulp blood compared with that of the red cells of the general circulation as due to the ability of the spleen to sift or detain the cells with smaller resistance. The general finding that the resistance of red cells to hypotonic saline increases following splenectomy, a fact which Orahovats corroborates, is not in accord with his explanation, for, obviously, if it is the function of the spleen to retain the more fragile cells then the resistance of the circulating red cells should not increase, but might be expected to decrease, after splenectomy.

On the basis of our results, we suggest that the spleen produces a substance or substances which have a direct effect on the red cell membrane, decreasing its resistance to various types of lysis, thus aiding its destruction. This explanation is similar to the one advanced by Botazzi (1894).

SUMMARY

1. It is shown that, in the guinea pig, simple abdominal incision or removal of organs produces an anemia of the same severity as that occurring after splenectomy.

2. The response of the erythropoietic system to a low pressure stimulus is similar in splenectomized and control animals, so the view that the spleen exerts an influence on the activity of the erythropoietic system does not appear to be substantiated in the guinea pig.

3. Animals exposed to the low pressure stimulus varying periods of time after splenectomy show a maximum loss of ability to destroy red cells 25 to 35 days subsequent to the operation.

4. This loss is due not only to a lessening of phagocytic activity because of the removal of a portion of the reticulo-endothelial system, but also to the increased resistance of the red blood cells, which attains a maximum value at about the same time as the maximum loss of red cell destroying power within the organism.

5. The organism, however, soon compensates and the rate of red cell destruction becomes normal because of an increase in the numbers and phagocytic activity of the reticulo-endothelial elements in other parts of the system, and also because some other part of the body has taken over the function of the spleen in decreasing the resistance of the red cells to destruction.

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ACID INHIBITION OF THE INTESTINAL AND INTRAGASTRIC CHEMICAL PHASES OF GASTRIC SECRETION

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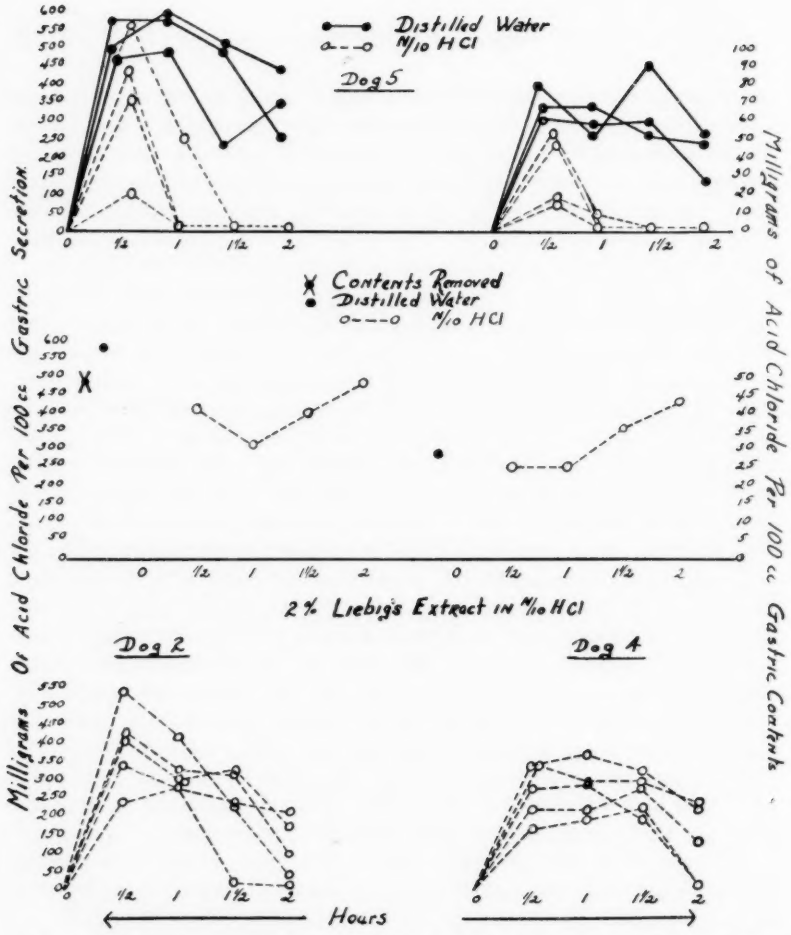
In a previous communication (1) it was shown that the *intragastric chemical phase* of acid secretion is progressively inhibited as the acidity of the gastric contents is increased. An acidity of the gastric contents approximating tenth normal hydrochloric acid was found to cause either complete or very marked inhibition of acid secretion in all dogs studied. This inhibitory effect was shown to operate largely, possibly solely, through intragastric mechanisms, since it was very marked in whole stomach pouches. In an earlier paper (2) it was shown that this inhibiting action of acid is not seen when histamine is used to excite gastric secretion, thus indicating that histamine is qualitatively or quantitatively (or both) different from the naturally occurring secretogogues present in Liebig's meat extract. In the present paper we wish to present data showing the effect of the acidity of the gastric contents first, on the intestinal phase of acid secretion and second, on the simultaneously combined intestinal and intragastric chemical phases of acid secretion.

METHODS. Five normal dogs were studied. All had been used in gastric studies over a period of 4 or 5 months and were thoroughly accustomed to the passage of the tube and the other manipulations. Very finely ground fresh beef liver was used to excite the intestinal phase. Liver was used because Kim and Ivy (3) have shown that liver extract exerts a much greater intestinal phase than meat extract, and also because when finely ground it can easily be washed from the stomach through an ordinary sized tube. Three hundred fifty grams of liver were fed by mouth from 5 to 6 hours before performing an experiment. Before starting the experiment the stomach was emptied as completely as possible and then lavaged with successive 300 cc. portions of distilled water warmed to approximately body temperature. The water was placed in the stomach and allowed to remain for 1 to 3 minutes and then removed and another 300 cc. portion introduced. This was repeated until the return was nearly clear and practically free from liver particles. The number of lavages needed varied from 5 to 7. Following removal of the last distilled

water lavage the stomach was given a final lavage with 300 cc. or more of the test fluid to be used. The volume, per cent of phenol red, and titratable acidity were always determined on this final lavage. The wet weight of the liver removed from the stomach was always determined; it varied from 12 to 158 grams and averaged 98 grams, thus showing that most of the liver had entered the intestine. Due to the care taken in passing the tube and to the previous training of the animals, there was no evidence of nausea or dislike for the repeated lavaging. Following removal of the final lavage the test solution was placed in the stomach and the experiment started. Both the fractional and the "Block" methods (2) of analysis were used, each being particularly suited to certain phases of the investigation. The test solutions placed in the stomach were *distilled water*, *tenth normal hydrochloric acid*, and *two per cent Liebig's extract in tenth normal hydrochloric acid*. Each test solution contained 15 mgm. of phenol red per liter. Gastric samples were removed every half hour for two hours and were analyzed for total chloride, neutral chloride and by difference the acid chloride, the titratable acid and the per cent of phenol red. Twenty per cent sodium tungstate and 1.33 normal sulfuric acid were used to clear the samples before determining the per cent of phenol red. The methods and calculations have been described in detail in previous papers (1, 2, 4).

RESULTS. Details of typical single experiments are shown in the figure and in table 2, while the average secretions under the various conditions are shown in table 1. In calculating the results shown in table 1 average curves for the total fluid, acid fluid and non-acid fluid were plotted on coördinate paper. The areas under the respective curves were determined by means of a standardized planimeter and converted into cubic centimeters of the various fluid fractions as previously described (1).

1. *Experiments with distilled water.* In these control experiments distilled water containing phenol red was placed in the stomach following the final lavage. Because of the rapid emptying of the stomach with distilled water, the "Block" method with 600 cc. each half-hour was usually employed. A typical experiment is shown in the upper third of the figure. The results are expressed both as milligrams of acid chloride per 100 cc. of gastric contents and per 100 cc. of secretion entering the stomach. The latter values are independent of the amount of water or other test solution remaining in the stomach and are, therefore, comparable in all experiments. Both methods of expression show that there was a high rate of acid secretion which continued for at least two hours. This secretion undoubtedly represents a combination of two phases—namely, the *intragastric chemical phase* which continued for some time after the liver had been removed from the stomach, and the *intestinal phase*. These control experiments show that the frequent lavaging just before starting the experiments did not exert an inhibitory effect on secretion as suggested by Ivy (5). Nine experi-



Upper third. Experiments on dog 5 showing the acidity curve 5.5 hours after 350 grams of liver *a*, when distilled water was placed in the stomach, and *b*, when tenth normal hydrochloric acid was placed in the stomach. On left side of figure the results are expressed as milligrams of acid chloride per 100 cc. of gastric secretion, while on right side they are expressed as milligrams of acid chloride per 100 cc. of gastric contents. The experiments are of the "Block" type.

Middle third. Experiments on an isolated stomach pouch 6 hours after feeding 350 grams of liver by mouth. The results show the acidity of the contents removed from pouch 6 hours after liver feeding, the acidity when distilled water was placed in the pouch for one-half hour, and the acidity for four one-half hour periods when tenth normal hydrochloric acid was placed in the pouch. Experiments were of the "Block" type. On the left the acidity is given as milligrams of acid chloride per 100 cc. of secretion and on the right per 100 cc. of contents.

Lower third. Experiments in which 2 per cent Liebig's extract in tenth normal hydrochloric acid was placed in the stomach 5.5 hours after 350 grams of liver. The results are expressed as milligrams of acid chloride per 100 cc. of gastric secretion. The experiments on dog 2 were the fractional type and those on dog 4 were the "Block" type.

TABLE 1

Shows the average secretions under the conditions listed in column 1

The average values were obtained as explained in text

TYPE EXPERIMENT	DOG 1	DOG 2	DOG 3	DOG 4	DOG 5
Distilled water after liver	Total fluid 111 cc. Acid fluid 67 cc. Non-acid fluid 44 cc. Per cent acid fluid 60 Block expts.		Total fluid 49 cc. Acid fluid 28 cc. Non-acid fluid 21 cc. Per cent acid fluid 57 Fractional expts.		Total fluid 88 cc. Acid fluid 67 cc. Non-acid fluid 21 cc. Per cent acid fluid 76 Block expts.
N/10 HCl after liver	Total fluid 80 cc. Acid fluid 17 cc. Non-acid fluid 63 cc. Per cent acid fluid 21 Block expts.	Total fluid 48 cc. Acid fluid 3 cc. Non-acid fluid 45 cc. Per cent acid fluid 6 Fractional expts.	Total fluid 42 cc. Acid fluid 8 cc. Non-acid fluid 34 cc. Per cent acid fluid 19 Fractional expts.	Total fluid 147 cc. Acid fluid 24 cc. Non-acid fluid 123 cc. Per cent acid fluid 16 Block expts.	Total fluid 88 cc. Acid fluid 12 cc. Non-acid fluid 76 cc. Per cent acid fluid 14 Block expts.
Aqueous 2 per cent Liebig's extract fasting		Total fluid 102 cc. Acid fluid 55 cc. Non-acid fluid 47 cc. Per cent acid fluid 54 Fractional expts.	Total fluid 125 cc. Acid fluid 65 cc. Non-acid fluid 60 cc. Per cent acid fluid 52 Fractional expts.		
2 per cent Liebig's extract in N/10 HCl fasting		Total fluid 40 cc. Acid fluid 10 cc. Non-acid fluid 30 cc. Per cent acid fluid 25 Fractional expts.	Total fluid 45 cc. Acid fluid 3 cc. Non-acid fluid 42 cc. Per cent acid fluid 7 Fractional expts.	Total fluid 87 cc. Acid fluid 25 cc. Non-acid fluid 62 cc. Per cent acid fluid 29 Block expts.	
2 per cent Liebig's extract in N/10 HCl after liver		Total fluid 62 cc. Acid fluid 28 cc. Non-acid fluid 34 cc. Per cent acid fluid 45 Fractional expts.	Total fluid 72 cc. Acid fluid 32 cc. Non-acid fluid 40 cc. Per cent acid fluid 45 Fractional expts.	Total fluid 144 cc. Acid fluid 63 cc. Non-acid fluid 81 cc. Per cent acid fluid 44 Block expts.	

TABLE 2

Experiments on dog 3

Group 1 shows the average secretion with aqueous two per cent Liebig's extract test meal. *Group 2* shows the inhibitory effect of N/10 hydrochloric acid on the intragastric chemical phase of acid secretion. *Group 3* shows the effect of tenth normal hydrochloric acid on the simultaneously combined intragastric chemical and intestinal phases of acid secretion. The first three experiments show very little evidence of inhibition, while the last two show definite inhibition.

GROUP	ACID CHLORIDE PER 100 CC. GASTRIC CONTENT	ACID CHLORIDE PER 100 CC. SECRETION	TOTAL FLUID	ACID FLUID	NON-ACID FLUID	TIME	REMARKS
	mgm.	*mgm.*				*hours*	
1	36	278	13	6	7	½	Average of 7 fractional experiments with 2 per cent aqueous Liebig's extract test meal. Dog fasting 24 hours
	104	386	27	17	10	1	
	159	306	52	27	25	1½	
	204	346	59	34	25	2	
2	10	0	4	2	2	½	Fractional experiments with two per cent Liebig's extract in N/10 HCl. Dog fasting for 24 hours
	0	0	7	0	7	1	
	0	0	12	0	12	1½	
	0	0	17	0	17	2	
	13	325	4	2	2	½	
	13	118	11	2	9	1	
	20	105	19	3	16	1½	
	0	0	26	0	26	2	
	15	500	3	3	0	½	
	3	33	9	0.5	8.5	1	
	19	111	17	3	14	1½	
	0	0	25	0	25	2	
	21	300	7	4	3	½	
	17	142	12	3	9	1	
	7	41	17	1	16	1½	
	8	33	24	1	23	2	
	0	0	7	0	7	½	
	0	0	11	0	11	1	
	0	0	24	0	24	1½	
	0	0	29	0	29	2	
3	71	506	14	12	2	½	Fractional experiments with two per cent Liebig's extract in N/10 HCl. 5½ hours after 350 grams liver
	63	262	24	11	13	1	
	106	272	39	18	21	1½	
	102	218	47	17	30	2	

TABLE 2—*Concluded*

GROUP	ACID CHLORIDE PER 100 CC. GASTRIC CONTENT	ACID CHLORIDE PER 100 CC. SECRETION	TOTAL FLUID	ACID FLUID	NON-ACID FLUID	TIME	REMARKS
	mgm.	*mgm.*				*hours*	
3	75	441	17	12	5	½	
	94	362	26	16	10	1	
	117	355	33	20	13	1½	
	114	335	34	19	15	2	
	50	556	9	8	1	½	
	53	312	17	9	8	1	
	68	296	23	11	12	1½	
	74	232	32	12	20	2	
	23	384	6	4	2	½	
	6	60	10	1	9	1	
	26	118	22	4	18	1½	
	0	0	24	0	24	2	
	35	436	8	6	2	½	
	43	269	16	7	9	1	
	33	138	24	6	18	1½	
	0	0	29	0	29	2	

ments were performed on 3 animals with identical results as shown by the average secretions in table 1.

2. *Experiments with tenth normal hydrochloric acid.* After the final lavage, tenth normal hydrochloric acid containing phenol red was placed in the stomach and samples removed every half hour for two hours. The results were strikingly uniform on all dogs and a typical result is shown in the upper third of the figure. There was practically always evidence of secretion during the first and occasionally during the second half-hour period. After this, the secretion dropped to zero and acid deficits were obtained due to neutralization of some of the acid in the test solution. The average results on the five dogs are shown in table 1. As explained under *methods*, the last lavage obtained before starting the experiment was analyzed as to volume, acidity and per cent of phenol red. The *total quantity* of acid in the last lavage could thus be compared with the *total quantity* of acid in the first sample of a "Block" experiment. This comparison showed that the high acidity of the first half-hour samples was due primarily to the secretion of acid and not to acid which had been left in the stomach. Twenty-four experiments were performed on five dogs with similar results.

The shape of the secretory curves in these experiments suggested the

following working hypothesis: At the beginning of the experiment two phases of acid secretion (intragastric chemical and intestinal) are in operation simultaneously and this combination of stimuli is too powerful to be inhibited by acid. After a short latent period the intragastric chemical phase is inhibited due to the presence of acid in the stomach as previously shown (1). The intestinal phase is not inhibited by acid in the stomach but continues until sufficient acid has entered the intestine where inhibition is brought about. This hypothesis which accounts for the initial secretion and the subsequent progressive inhibition was tested in the remaining experiments.

3. *Effect of acid on the intestinal phase in a stomach pouch.* The pouch consisted of all of the stomach except a narrow tube alone the lesser curvature which served to connect the esophagus with the duodenum. The animal was fed 350 grams of finely ground liver 6 hours before the experiment. The accumulated secretions in the pouch were removed and analyzed and 200 cc. of distilled water containing phenol red were introduced and allowed to remain for ½ hour. As seen in the middle third of the figure, the secretions removed and also the control distilled water experiment both show that the intestinal phase of acid secretion was in operation. Two hundred cubic centimeters of N/10 hydrochloric acid containing phenol red were next introduced and allowed to remain for ½ hour. After removal of this, a second 200 cc. portion was introduced and allowed to remain for ½ hour. This was continued until four half-hour samples had been obtained. In the figure the results are plotted both as milligrams of extra acid chloride per 100 cc. of secretion entering the stomach and per 100 cc. of gastric contents. It is evident from these experiments that the intestinal phase of acid secretion is not inhibited by the presence of acid in the stomach. From this it follows that the inhibition of the intestinal phase seen in the intact animal must be due to the acid which leaves the stomach and enters the intestine.

4. *The effect of acid on the simultaneously combined intragastric chemical and intestinal phases.* In these experiments the animals were fed the usual amount of liver and 5.5 hours later the stomach lavaged practically clean. A meal consisting of 2 per cent Liebig's extract in tenth normal hydrochloric acid was then introduced and half-hour samples obtained according to either the "Block" or fractional methods for the next two hours. A complete set of experiments on dog 3 is shown in table 2 and the experiments on the remaining dogs are shown in the lower third of the figure. The majority of these experiments showed a high rate of secretion which was usually maintained for about one and one-half hour, after which there was often evidence of inhibition. In a few instances inhibition was marked throughout the experiment. As shown in table 1 the *average* result was a fairly high rate of acid secretion which is strikingly different

from the *average* result when tenth normal acid alone was placed in the stomach. These experiments show that when the two phases are simultaneously combined, the resultant stimulus for secretion will, in the majority of instances, overcome the inhibiting effect of the acid and give rise to a fairly high rate of secretion.

DISCUSSION. The experiments described above appear to justify the following conclusions: The presence of tenth normal hydrochloric acid *in the stomach* does not inhibit acid secretion due to the intestinal phase. As the acid leaves the stomach and enters the intestine, inhibitory mechanisms are set up from the intestine which inhibit acid secretion due to the intestinal phase. When the *intragastric chemical* and *intestinal* phases are in operation simultaneously, the combined stimulus is usually too powerful to be inhibited by acid in the stomach and intestine, and in the majority of instances a fairly high rate of acid secretion will result.

While this work was in progress, a paper appeared by Day and Webster (6) in which they showed that introduction into the duodenum of 0.25 per cent hydrochloric acid or diluted gastric juice will inhibit gastric secretion due to the intestinal phase. Their conclusions were based on a study of the *volume* of the gastric secretions, not on the *acidity*. More recently Griffiths (7) has shown that the introduction of hydrochloric acid directly into the duodenum will inhibit the secretion of acid in response to an alcohol test meal. Neither of the above investigators specifically investigated the effect of acid inhibition on the simultaneously combined intragastric chemical and intestinal phases.

The results reported in this and a previous paper (1) are sufficiently conclusive to justify the formulation of a theory concerning the rôle of acid inhibition in the regulation of gastric acidity. This theory is as follows: Following the ingestion of food, the partially digested food products evoke acid secretion (intragastric chemical phase) resulting in an initial rapid rise in the acidity of the gastric contents. When the acidity of the gastric contents approaches 0.06 normal (60 clinical units), inhibitory processes are set in action which operate, within the stomach, to decrease the rate of acid secretion due to the intragastric chemical phase. Between 0.06 and 0.10 normal, this inhibitory mechanism becomes increasingly active and at 0.10 normal will either completely inhibit or very markedly decrease the rate of secretion. Thus, even in the absence of duodenal regurgitation this mechanism could prevent an excessive rise in the acidity of the gastric contents. If the intestinal phase of acid secretion begins while food is still in the stomach or while the intragastric chemical phase is still in operation, the simultaneous combination of the two phases will usually be sufficiently potent to prevent inhibition of acid secretion by the entrance of acid chyme into the intestine. As the intragastric chemical phase declines due to emptying and wearing off of the stimulus in the stomach,

the intestinal phase would tend to maintain a high rate of acid secretion in the empty stomach, which if not checked, might continue for many hours as is seen in whole stomach pouch preparations with the esophagus anastomosed to the duodenum. However, the passage of the acid gastric juice from the practically empty stomach into the intestine will inhibit acid secretion due to the intestinal phase *alone* and acid secretion will decline to the resting or fasting level. By means of these acid inhibitory mechanisms, it is theoretically possible for gastric acidity to be adequately regulated in the absence of duodenal regurgitation.

Although exact details are lacking, it is obvious that an elevation of the threshold for acid inhibition in either the stomach or the intestine could result in hyperacidity (hypersecretion) during digestion or prolonged hypersecretion in the empty stomach (prolonged intestinal phase). Lowering of the threshold could account for certain types of hyposecretion. All of these alterations in acidity could theoretically occur independent of changes in duodenal regurgitation.

It is interesting to note that the failure of acid to inhibit secretion when histamine is used as a stimulant (2) is possibly due to the fact that histamine is too powerful a stimulus to be controlled by acid inhibition, approximating or exceeding a simultaneous combination of the intragastric chemical and intestinal phases.

SUMMARY

1. The intestinal phase of acid secretion is not inhibited by the presence of tenth normal hydrochloric acid in the stomach. As the acid leaves the stomach and passes into the intestine, the intestinal phase is promptly and completely inhibited.

2. When the intestinal and intragastric chemical phases are simultaneously combined, the presence of tenth normal hydrochloric acid in the stomach and intestine is usually not able to inhibit acid secretion.

3. A theory of the regulation of gastric acidity by acid inhibition is presented. It is pointed out that shifts in the threshold for acid inhibition may be the cause of certain abnormalities of acid secretion.

4. By means of acid inhibition, it is theoretically possible for gastric acidity to be adequately regulated in the absence of duodenal regurgitation.

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THE INFLUENCE OF OIL ENEMAS ON COLON MOTILITY IN THE DOG

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Templeton and Lawson (1) described the motility of the dog's colon as recorded by the tandem balloon technique. Later (2) they reported that doubling the usual balloon pressure or direct electrical stimulation of the colon mucosa above the internal anal sphincter had very little effect on the circular activity.

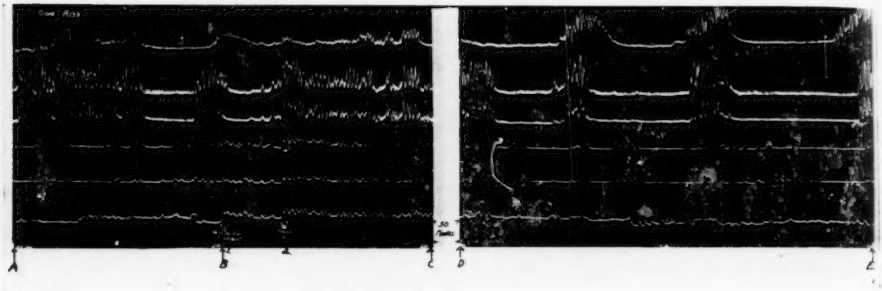
These observations on the reaction of the colon to mild stimuli and the general unanimity of opinion regarding the blandness of oil suggested the oil enema as a logical beginning for studying the influence of intralumenal injections on the colon motility. Montague (3) observed that the chief use of oil was to relax the intestinal musculature and soften scybala or lubricate the bowel when desired. Friedenwald and Feldman (4) found no evidence of pathological changes in the dog's colon following prolonged use of cottonseed oil enemas.

To secure the maximum effect, according to Sollmann (5), an enema must be used in a fairly large quantity. However, Lewis (6) warned against the use of too large a quantity because of the danger of paralysis to the colon. In preliminary observations we found that oil enemas in 100 and 200 cc. quantities would elicit defecation in trained animals 60 to 75 per cent of the times injected. On the other hand these quantities would permit retention without leakage or apparent discomfort over long periods of time.

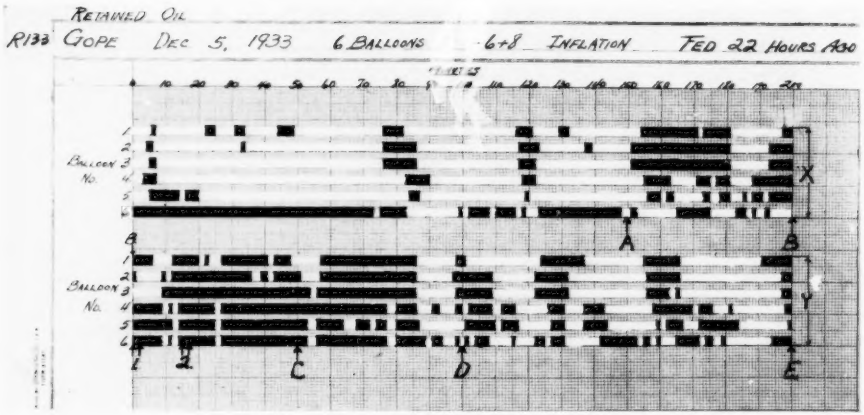
We recorded colon motility by the tandem balloon technique, employing two sets of three balloons each (7). Only well trained unanesthetized dogs, on which cecostomies had been prepared at least 30 days previously, were used. One set of balloons was inserted into the proximal colon by way of the cecostomy. The distal set was inserted by way of the anus. Without disturbing the animals, enemas were injected through the free end of a rubber tube (3/16 inch in diameter) attached to the distal set between the middle and upper balloons.

The usual method of showing alterations in motility has been to present a picture of the tracings most representative. This method was departed

from (fig. 1) to obtain a numerical representation of activity and secure data with which to summarize results. The active and quiet periods were



Part I



Part II

Fig. 1. Part I. Excerpts from a six balloon tracing.

A to *B*, 50 minutes of the control period; *1*, injection of 100 cc. of oil; *2*, repeat 1; *B* to *C*, 50 minutes of following oil injections; *C* to *D*, 50 minutes of tracing omitted; *D* to *E*, last 100 minutes of the tracing.

Part II. Diagrammatic graph of entire six balloon tracing of which excerpts of original are given in part I.

X, first 200 minutes of the tracing (control period); *Y*, second 200 minutes of the tracing. *A* to *B*, corresponds to *A* to *B* in part I; *1* and *2*, represents *1* and *2* in part I. *B* to *C*, corresponds to *B* to *C* in part I; *C* to *D*, corresponds to *C* to *D* omitted in part I; *D* to *E*, corresponds to *D* to *E* in part I.

first diagrammatically transferred to graph paper. To do this, two arbitrary rules were strictly followed. First, the gut was said to be active

if a contraction or contractions continued for one minute or more. Second, the gut was considered quiet if two minutes or more intervened between contractions. By this method the active and quiet periods were pictured in sharp contrast. From the sum of the minutes of activity the percentage of time during which the gut was active or quiet was computed for each 50 minute period in the experiment. No attempt was made to study the degree of activity as indicated by the height of individual contractions.

In the colon periods of activity recur at more or less regular intervals (fig. 1, part I). Variations in this regularity, however, make a forecast of the exact beginning or duration of a period impossible. The degree of activity recorded from six balloons may change 100 per cent any instant or may continue at the same level for an unpredictable time with very little variation. The independence of each segment of the gut makes any degree of change in the activity possible. In one 50 minute period all segments may be active giving a computed activity of 100 per cent. During the next period only one half of the segments may be active, or all of the segments may be active only one half of the time giving a computed activity of 50 per cent. It is possible, but seldom occurs, for all segments to be quiet throughout a 50 minute period. This susceptibility to abrupt changes in motility makes the trend of motility in a long experiment more important than the activity observed at any one instance.

The trend of motility for any animal may be fairly constant from day to day but may differ greatly from that of another animal. Therefore, the same animal on which the effect of a substance is to be studied must have many control experiments. These controls should be made at frequent intervals within the course of an investigation. In addition to the control experiments a control period should precede the introduction of the experimental factor and be of sufficient duration to allow an observation on the trend indicated.

The influence of oil on colon motility was considered from two points of view. First, the effect associated with retention. Second, the residual effect following expulsion. The first procedure included experiments run 400 minutes without removing the balloons. These were called the continuous experiments. The second procedure included those experiments in which the balloons were removed after 200 minutes, the dogs allowed freedom outdoors and after an interval of 30 minutes, the experiments resumed for another 200 minute period. These were called the interrupted experiments. If enemas were injected, they were introduced after 200 minutes of the experiment had been completed in both the continuous and interrupted experiments.

The continuous group consisted of 69 experiments on 4 dogs. Between the first and second 200 minute periods 18 of these received 100 cc. enemas of white mineral oil, 27 received 200 cc. enemas and the remaining 24

were continued as controls without injections. The oil was administered slowly and in broken doses in order to obviate as much as possible the mechanical effect of sudden distention. One half of the desired quantity was injected at the rate of 50 cc. per minute and between 10 and 15 minutes later the remainder was given.

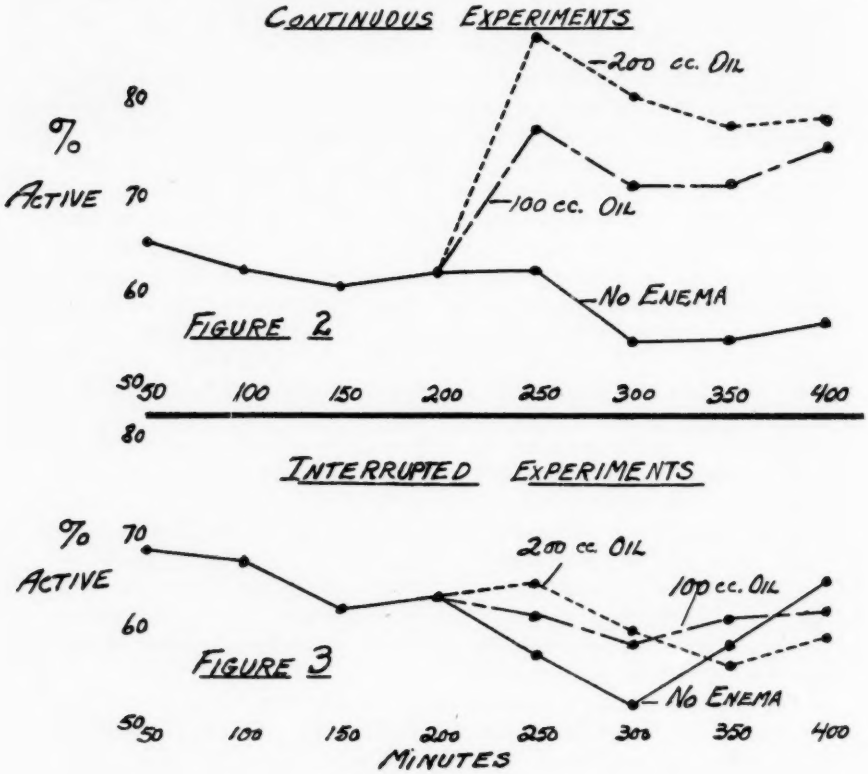


Fig. 2. See text for explanation.
Fig. 3. See text for explanation.

The average colon activity for all continuous experiments during the first 200 minutes (i.e., the control period) varied from 60 to 65 per cent (fig. 2). The activity during the second 200 minutes in those experiments in which no enema was given varied between 50 and 64 per cent. The activity during the second 200 minutes in those experiments in which 100 cc. oil enemas were retained increased to between 71 and 77 per cent, the maximum being reached within 50 minutes after giving the

enema. These figures represent an increase over the control group of 15 to 18 per cent. The retention of 200 cc. of oil gave the same qualitative effect as when 100 cc. enemas were retained. The activity was increased to between 77 and 86 per cent, the maximum being reached within 50 minutes after giving the enema. Thus the retention of 200 cc. of oil resulted in an increase of activity, 21 to 25 per cent over the control group.

In the interrupted group of experiments only those animals which defecated in the 30 minute interval between the first and second 200 minutes of the experiments were considered. This group included 53 experiments on the same dogs used in the continuous study. Of these 18 received 100 cc. enemas of white mineral oil, 17 received 200 cc. enemas and the remaining 18 were used as controls without injections. The same method of administering the oil was followed as in the continuous group.

The average activity of the entire interrupted group during the first 200 minutes varied from 62 to 68 per cent (fig. 3). The activity during the second 200 minutes in the control group varied from 52 to 65 per cent. The activity during the second 200 minute period in those experiments in which enemas were given varied from 56 to 64 per cent. The results were practically the same whether 100 or 200 cc. quantities were given, the activity following both being slightly greater than that of the control group.

SUMMARY

1. A method is presented which graphically contrasts the active and quiet periods of gut motility.

2. Colon motility is quantitated with reference to the percentage of time during which activity occurs.

3. Colon activity is augmented for at least 200 minutes by the retention of 100 cc. or 200 cc. oil enemas.

4. Colon activity is not greatly altered by 100 or 200 cc. oil enemas, if evacuation occurs shortly after their administration.

The authors are indebted to Dr. A. J. Carlson under whose direction this work was conducted.

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THE CONTROL OF THE LARGE INTESTINE BY THE DECENTRALIZED INFERIOR MESENTERIC GANGLION

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There is satisfactory evidence for independent activity in the sympathetic ganglia of the frog (Boshamer, 1925; Hermann and Guiran, 1934), and perhaps less conclusive evidence for the crayfish (Prosser, 1934) and the water-beetle (Adrian, 1931). Data on mammalian ganglia, however, are either negative or inconclusive. Hermann and Morin (1935) were unable to demonstrate that the decentralized sympathetic ganglia had any influence on vascular tone in the hind limbs of the dog. Brooks (1935) was unable to show that the sympathetic ganglia of the cat are stimulated by hemorrhage. However, Govaerts (1935) has reported from acute experiments on cats that excision of the stellate ganglion five to twenty days after decentralization produced further slowing of the heart. More recently Evans (1936) has recorded activity in the vesical plexus of the cat in acute experiments ten days after decentralization. Evidence has also been offered for continued inhibition of the large intestine in the cat by the acutely decentralized inferior mesenteric ganglion (Garry, 1933), and for a complex motor control of the large intestine by this ganglion following acute decentralization in the dog (Lawson, 1934).

Evidence for independent ganglionic activity in mammals thus rests upon acute experiments, in which operative trauma and exposure may have served as stimuli. Adrian, Bronk, and Phillips (1932) concluded that trauma and exposure were the cause of bursts of efferent impulses which they occasionally observed in the hypogastric nerve of the cat following acute decentralization of the inferior mesenteric ganglion.

In the present study an attempt has been made, using spontaneous motility in the colon of the unanesthetized dog as an index, to obtain further data on activity in the inferior mesenteric ganglion. The data on ganglionic activity are thus indirect, but avoid the criticism to which direct recording of action potentials, with exposure of the ganglion, is liable.

METHODS AND RESULTS. Colon motility was recorded with a system of four air-filled balloons as previously described (Lawson and Templeton,

1931), balloon I lying near the cecostomy and balloon IV in the anal canal at the level of the internal anal sphincter. Pressure within the balloons was recorded with bromoform manometers. The motility of the third hour following insertion and inflation of the balloons was taken as a sample from each tracing. This sample was divided into four-minute periods in each of which maximum and minimum pressure was read in each balloon. The pressure limits determined in this manner were plotted for each animal, and averages of the upper and the lower limits taken as a quantitative index for the animal.

This method of handling the data obviates the difficult distinction between tone changes and contractions. Although it does not derive either average tone or average contraction height, it shows accurately the height and the frequency of maximum contractions and minimum tone levels throughout the period of observation. Since, further, the method offers a rough index to average tone and average contraction height, the lower limits of motility will be referred to as "tone," the upper limits as "contraction height," and the difference as "contractility."

In three animals a study was made of the effect of decentralization of the ganglion. After a thirty-day control period during which daily records were made, the spinal rami of the ganglion were divided aseptically and the animals allowed to recover, daily records being made as before for the following two to four weeks. The average pressure limits before and after ramisection are shown in figure 1. Tone levels were lowered in the proximal colon and the anal canal, and raised in the middle colon. Contraction height was not uniformly affected except in the distal colon, where there was an increase roughly proportional to the increase in tone level.

The effect of removal of the previously decentralized ganglion was studied in six animals. Functional ganglionectomy was accomplished by division of the hypogastric and the lumbar colonic nerves, anatomical removal of the ganglion being impracticable without ligation of the inferior mesenteric artery. In three animals ganglionectomy was done two to four weeks following ramisection, in the remaining three, four to six months after ramisection. The results were essentially the same in the two groups. The effect on pressure limits throughout the colon is shown in figure 1. The height of contractions was increased, most markedly in the distal colon and anal canal (average increase for all animals 80 per cent), and least markedly in the proximal segment (average increase for all animals 35 per cent). In only one dog (dog 3, segments III and IV) did an increase fail to appear. Observations on this animal had to be discontinued within four days after the operation, and are included only for the sake of completeness. Tone levels were not uniformly affected by ganglionectomy, in some instances being lowered.

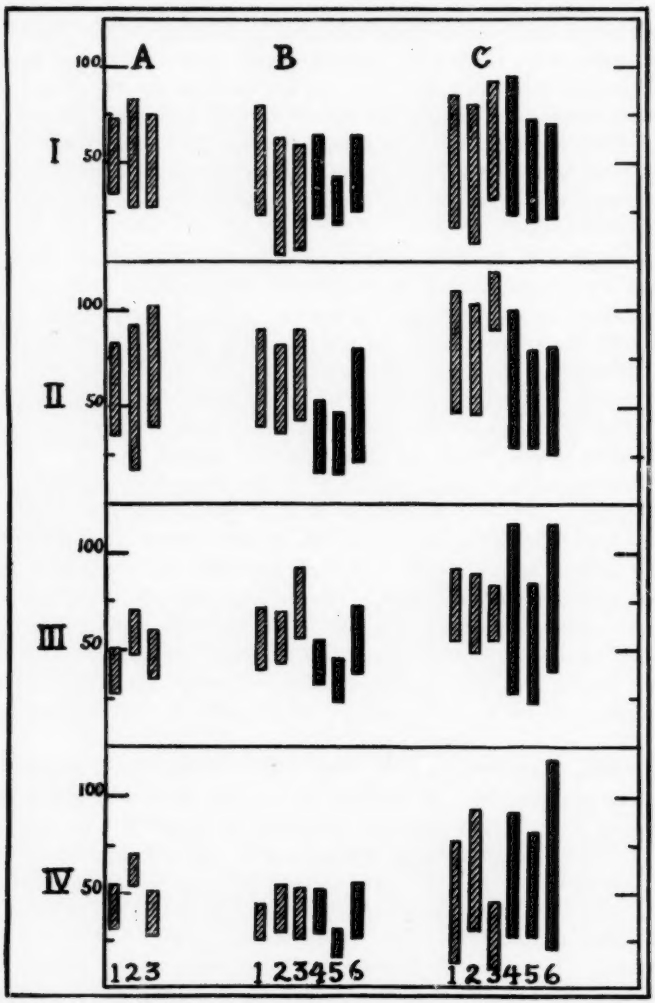

Fig. 1. Average limits of pressure (millimeters bromoform). A—Preoperative control. B—After ramisection. C—After ganglionectomy. The colon segments are numbered on the left: I—Proximal colon, II—middle colon, III—distal colon, IV—internal anal sphincter. Averages for each of three dogs are given in A, six in B and C, the shaded rectangles indicating the animals on which preoperative controls were obtained. The number of the animal is given at the bottom below the corresponding set of averages.

The increase in the amplitude of contractions following ganglionectomy constitutes a striking change in the motility of the distal colon and anal canal. Figure 1 shows the marked difference between contractility in the proximal and the distal segments before ganglionectomy, and the loss of this difference after ganglionectomy. As a qualitative modification, not shown by this method of tabulation, peristaltic waves appeared in the distal segments much more frequently after ganglionectomy.

Records made within twelve hours after ganglionectomy showed the increase in contractility fully established. No uniform progressive change was noted thereafter, although two of the dogs were studied for nine

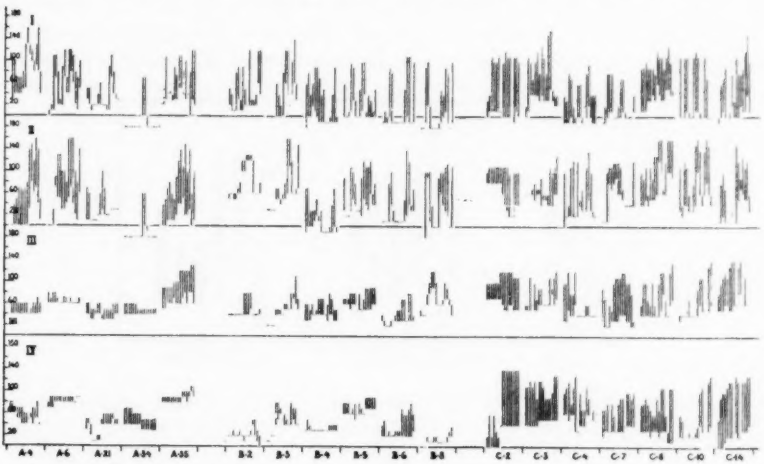

Fig. 2. Limits of pressure from daily records (millimeters bromoform). Dog 2. Colon segments, as in figure 1, are numbered on the left. At the bottom, below each day's limits, are given the period of the experiment (A, B, and C as in fig. 1), and the number of days since the beginning of the period. Ganglionectomy was done fifteen days following ramisection.

weeks following ganglionectomy. Figure 2 shows the relatively stationary character of the modification throughout the period of degeneration of the divided nerves.

In order to follow the course of degeneration, small doses of epinephrine (2 to 4 cc. of 1:50:000 solution) were given intravenously during each tracing, usually during the fourth or fifth hour of the tracing. Figure 3 shows the average height of the excitatory response and of the secondary inhibitory response (Lawson, 1934) in the internal anal sphincter for each animal. Ramisection increased the height of the excitatory phase in all three animals with preoperative controls. Subsequent ganglionectomy either reduced the excitatory response (three animals) or produced no

significant change (three animals). Secondary inhibition, however, which was not significantly affected by ramisection, was more profound in all six animals following ganglionectomy. Responses outside the sphincter area were too inconstant for tabulation.

The permanent changes in the response of the sphincter to epinephrine following interruption of the lumbar outflow at the two levels are thus the same as those previously reported to occur in acute experiments. No progressive change was observed following interruption at either level.

Discussion. The validity of these data as proof of independent ganglionic activity depends upon a clear-cut demonstration that our preliminary operation affords complete decentralization. It must be granted that a few remaining preganglionic fibres, by virtue of multiple ganglionic connections (Bishop and Heinebecker, 1932), might suffice for

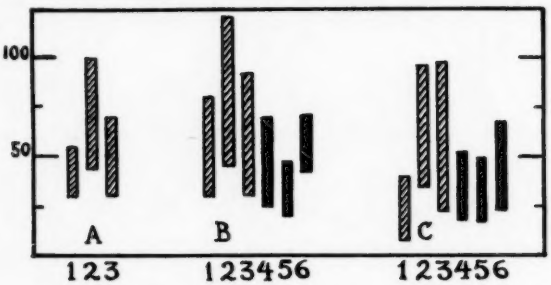

Fig. 3. Average height of the excitatory and the inhibitory response to epinephrine in the internal anal sphincter. The height of the excitatory response (maximum pressure following injection) is given by the upper extent of each rectangle, the depth of the inhibitory response (minimum pressure following injection) by the lower extent. The construction of the figure is the same as in figure 1. Each rectangle represents twenty to forty responses.

nearly complete control of the colon. The surgical technique employed leaves only the hypogastric and the lumbar colonic nerves, and the wall of the inferior mesenteric artery, which was cauterized with phenol, as avenues by which preganglionics might enter the ganglion after ramisection. Careful examination of the ganglion and the surrounding mesentery by the pyridine-silver method in one animal after the second operation failed to show any fibres entering the ganglion.[1] Results on this animal had been typical.

Both spontaneous activity and the response to epinephrine are changed by ramisection, but the modifications are not in the same direction as those following ganglionectomy. Contractility in the lower colon is increased by ganglionectomy, but not by ramisection, the principal effect of which seems to be on tone. The excitatory response to epinephrine is increased

[1] The authors are indebted to Dr. S. E. Johnson for the histological data.

by ramisection, the inhibitory response by ganglionectomy. These findings point to the residence of more or less specific functions in the ganglion.

It is significant that ganglionectomy produces immediate changes in the tone and irritability of the colon (Garry, 1933; Lawson, 1934) which the present data show persist without further modification for several weeks. Hampel (1935) has shown that sensitization of the nictitating membrane to epinephrine increases progressively following denervation, reaching a maximum within about two weeks. The absence of any progressive increase in contractility in our series during the period of observation suggests that the change following ganglionectomy is not due to sensitization of the denervated colon to locally produced or circulating excitants. The absence of progressive change in the response to epinephrine supports this conclusion. The remaining interpretation, which is compatible with the data on both the acutely and the chronically ganglionectomized animals, postulates nervous activity in the ganglion in the absence of central connections.

SUMMARY

Division of the spinal rami of the inferior mesenteric ganglion decreases tone in the anal canal and the proximal colon, and increases tone in the middle colon in unanesthetized dogs. Contractility is unaffected. Subsequent removal of the ganglion has no constant effect on tone, but markedly increases contraction height, especially in the anal canal and the distal colon.

The excitatory response of the internal anal sphincter to epinephrine is increased by ramisection, the inhibitory response by subsequent ganglionectomy.

The control of the colon by the decentralized ganglion seems to be due to nervous activity in the ganglion, rather than to persistence of the postganglionic fibres, since the effect of ganglionectomy is fully established within a few hours.

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THE EFFECT OF A LYSINE DEFICIENT DIET ON THE ESTROUS CYCLE[1]

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While the rôle of lysine in growth has been carefully studied by Osborne and Mendel (1912), (1916), knowledge concerning the relation of this amino acid to the reproductive processes is meager. Osborne and Mendel (1912) reported that feeding a diet deficient in lysine does not impair the capacity of the animal for reproduction. However, this statement was based on the performance of a single animal. Evans and Bishop (1922) noted a disturbance in the estrous cycle when young rats were fed on a diet containing wheat as the sole source of protein. Courrier and Raynaud (1932) showed that there was cessation of the estrous cycle when rats were maintained on a diet containing 18 per cent gliadin, which was the sole source of protein except for the small amount that may have been present in the vitamin supplements. Estrus occurred within 3 days after implantation of an hypophysis of a normal rat into an animal that had ceased to cycle. It is now a well established fact that estrus may be brought about by the administration of various endocrine preparations. Nevertheless, these workers concluded that lysine was necessary for ovulation.

In order, if possible, to establish beyond doubt the effect of a lysine-deficient diet on the estrous phenomena we have fed 6 groups of rats on different diets using gliadin as the chief source of protein. Unnecessary details are avoided and the salient results presented by selecting 3 typical groups from the series. The results for each of these groups will be discussed separately and comparisons made of the sexual behavior of the animals on the different dietary regimens. The animals used were fed a standard stock diet until they reached sexual maturity and presented a normal estrous cycle. The estrous cycle was followed by means of daily microscopic examination of the vaginal smear.

EXPERIMENTAL. The diets employed are presented in table 1. Gliadin was prepared by extracting wheat gluten with 70 per cent ethyl alcohol.

[1] A preliminary report of a portion of this work was presented at the twenty-ninth annual meeting of the American Society of Animal Production at Chicago, November 1936.

A concentrate of hexone bases was prepared from gelatin hydrolysate by the electrical transport method described by Foster and Schmidt (1923). The water from the dialysate was evaporated off over a steam bath and the residue dried in a vacuum oven. The following amounts of vitamin supplements were fed individually each day.

Yeast extract	0.5 ml.
Liver extract	0.3 ml.
Cod liver oil	3 drops
Wheat germ oil	3 drops

The diet contained adequate vitamins as evidenced by the fact that growth was resumed when lysine was added.

Six female rats averaging 184 grams in weight at 65 days of age were placed on diet 3-A, containing 10 per cent gliadin. Within 2 weeks all of

TABLE 1

Percentage composition of diets

	DIET NO.				
	3-A	3-D	3-F	3-F-b	3-G
Gliadin	10.0	18.0	15.0	14.6	17.4
Corn starch	74.0	66.0	66.0	66.4	66.0
Butter fat	12.0	12.0	12.0	12.0	12.0
Salts—F. R. L. mixture*	4.0	4.0	4.0	4.0	4.0
Hexone concentrate			3.0	3.0	
d-Lysine dihydrochloride					0.6
Nitrogen per cent	1.70	2.79	2.98	2.88	2.77

* Hawk and Bergeim (1931).

the animals showed irregular or complete cessation of the estrous cycles. The performance of these animals is summarized in figure 1, by means of a composite growth curve and a graphical presentation of their behavior in respect to the estrous cycle. Usually the animals showed 2 or 3 normal cycles after being placed on the experimental diet and then abruptly ceased to cycle. Rat B4027 presented two normal cycles, then a period of 12 days elapsed before the third estrus occurred. Asdell and Crowell (1935) in their study of the effect of retarded growth on the estrous cycle considered intervals of more than 10 days abnormal. This may be an arbitrary figure, but it would seem that intervals exceeding this are abnormal and may be attributed to the dietary regimen. Concurrently with placing the animals on the experimental diet there was an average loss in weight of 14 grams.

The animals were continued on the diet containing 10 per cent gliadin

for a period of 80 days. After the initial weight decrement during the first 2 weeks, their average weight remained almost constant throughout the remainder of the period. There was no tendency for the estrous cycles to be resumed and there is no reason to believe that the performance of the animals would have changed had they been continued longer on diet 3-A.

At the end of the 80 day period the animals were changed to diet 3-D, thereby increasing the gliadin content from 10 to 18 per cent. While the protein was previously deficient both quantitatively and qualitatively, on the higher level it was incomplete only in the latter respect. During

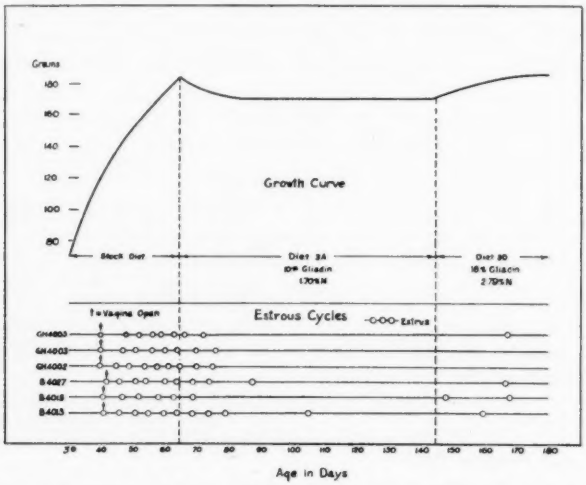

Fig. 1. Growth curve and estrous behavior of rats on diets containing 10 per cent and 18 per cent of gliadin as the sole source of protein. Estrus is indicated by a circle.

the 40 days that the animals were carried on this diet there was a slight weight increment. Three of the animals came into estrus once and no. B4015 came into estrus twice at intervals of 20 days each. From the behavior of these 3 animals on the 18 per cent gliadin level it might be deduced that there was a tendency for the estrous cycles to be resumed. The possibility that a diet containing 18 per cent gliadin as the sole source of protein may permit regular estrous behavior has been studied in a second group of rats.

For this purpose 6 sexually mature rats averaging 154 grams at 55 days of age were placed on diet 3-D. The sexual behavior of these animals is presented graphically together with a composite growth curve in figure 2.

After placing the animals on the gliadin diet complete cessation of the estrous cycle occurred. In no case did more than 2 normal cycles occur; no. G4551 came into estrus a third time after a period of 13 days had elapsed. The animals were carried on this diet for a period of 44 days. There was a decline in weight when the animals were placed on the experimental diet, but after adapting themselves to the new regimen there was an increment in weight so that at the end of the 44 day period the animals were on an average 16 grams heavier than they were at the beginning of the period. Notwithstanding this slight increase in weight there was

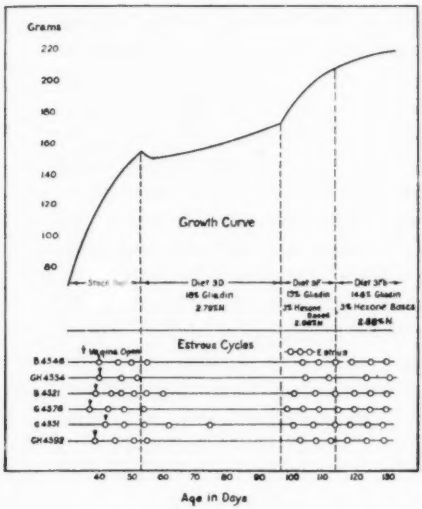

Fig. 2

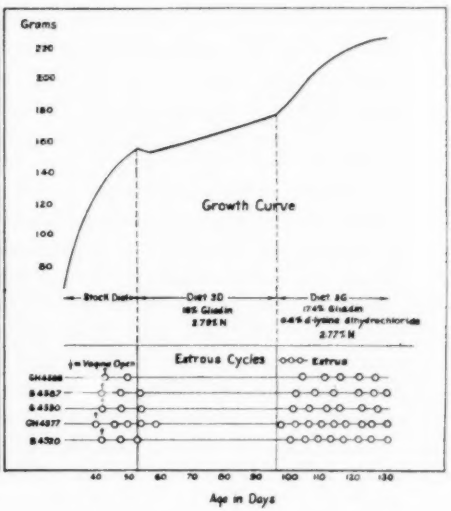

Fig. 3

Fig. 2. The effect of adding a preparation of hexone bases to a diet containing gliadin as the sole source of protein. Estrus is indicated by a circle.

Fig. 3. The effect of adding 0.6 per cent of *d*-lysine dihydrochloride to a diet containing gliadin as the sole source of protein. Estrus is indicated by a circle.

complete cessation of the estrous cycle on the diet containing 18 per cent gliadin.

Three per cent of the hexone preparation was then added to the diet replacing an equivalent amount of gliadin. Subsequently the gliadin was further reduced by 0.4 per cent in order to maintain the nitrogen content of the diets at approximately the same levels. From figure 2 it is seen that with the addition of the hexone preparation to the diet there was a marked increase in the rate of growth. Concurrently with the increase in growth normal estrous cycles were resumed. Within 8 days all of the animals came into estrus, and except for no. GH4534, continued

to present normal cycles thereafter. In this single case more than 10 days elapsed between periods 2 and 3, the other periods being normal. Had the animals been continued longer on this diet it is likely that no. GH4534 would have presented a normal estrous picture. All of the other animals showed 6 or more normal cycles each during the 34 days that the preparation of hexone bases was fed. These results would indicate that lysine was the deficient factor causing aberration of the estrous cycle. However, the possibility that arginine and histidine or impurities in the preparation of hexone bases might be factors in restoring the estrous cycle cannot be precluded.

To test the assumption that lysine was the limiting factor causing cessation of the estrous cycle on the gliadin diet a group of 5 rats was maintained on diet 3-D for a period of 44 days. These animals were 53 days of age at the time they were placed on the experimental diet and while they were sexually mature in respect to the estrous cycle, they were immature from the standpoint of growth, their average weight being 154 grams. Figure 3 presents graphically the behavior of the animals in respect to the estrous cycle together with a composite growth curve. As in the 2 preceding groups these animals increased in weight on the gliadin diet. There was a slight initial decrement in weight, but at the end of the 44 day period they weighed on the average 22 grams more than at the beginning. The fact that there was complete cessation of the estrous cycle concomitantly with the weight increment is of particular interest. Evans and Bishop (1922) observed an aberration of the estrous cycle on various inadequate nutritional regimens. On the other hand, Asdell and Crowell (1935) concluded from their studies of the effect of retarded growth on the sexual development of rats, that when additional energy is supplied there is no competition between the growth and sexual functions.

The striking results of adding 0.6 per cent of *d*-lysine dihydrochloride to the diet is shown in figure 3. There was a marked increase in the rate of growth and concurrently normal estrous cycles were resumed. During the 35 days that the animals received diet 3-G containing the *d*-lysine dihydrochloride 5 or more normal cycles were presented by each rat. The change in the estrous behavior upon the addition of lysine is very striking and clearly demonstrates that a deficiency of this amino acid causes irregular or complete cessation of the estrous cycle in the rat.

The results of these studies corroborate the findings of Evans and Bishop (1922) that complete cessation of the estrous cycle may occur concurrently with limited weight increments. Our data would indicate that in the immature animal the demands for growth are satisfied before those incident to the estrous cycle. The possibility that lysine may play a specific rôle in the reproductive processes cannot be precluded on the basis of the work reported in this paper. However, there would seem to

be no doubt that full reproductive capacity is reached only when the nutritive environment is complete, and that malnutrition inhibits the optimal functioning of the reproductive processes of the organism.

SUMMARY

A diet containing 18 per cent gliadin as the main source of protein causes cessation of the estrous cycle in the rat. Since the addition of 0.6 per cent of *d*-lysine dihydrochloride to the diet restores the estrous cycle, it is apparent that a deficiency of lysine is responsible for the aberration in the sexual behavior. There may be cessation of the estrous cycle and concomitantly a limited amount of growth. Thus it appears that the nutritive demands for growth may be satisfied before those for the estrous cycle.

Acknowledgments are made to Dr. G. H. Hart for suggesting this problem, and to him and Dr. H. Goss for their continued interest and encouragement throughout the course of the work.

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SOME NEURAL COMPONENTS OF THE VISUAL RESPONSE[1]

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There are many divergent views concerning the integration of monocular visual responses into the binocular response. Also there is much difference of opinion regarding the nature of light and dark adaptation and its relation to true visual fatigue.

We have approached the study of the first subject by the use of a method which had been previously applied by one of us to the study of the olfactory process. The studies of vision led us to make certain comparisons between monocular and binocular vision. The conclusions drawn from these comparisons appear to be significant and have a bearing upon the neural correlations of the visual process. The studies were made in a dark room with red light, the wave length of which was beyond 610$\mu\mu$ of the spectrum. The test objects consisted of small white squares, 1 mm. to 18 mm. in size, on a black background, viewed through a red filter (Wratten F 29), and illuminated by the light of a 30-Watt lamp at 1 meter distance from the test object. The smallest object that could be seen as a bright spot from a distance of 3.75 meters was taken as the threshold acuity object for brightness. The thresholds for each eye and for the two eyes together were first established. The eye or eyes were then exposed to a brighter red light (from a 30-Watt bulb 40 cm. from the eye) for ten seconds, and the time which elapsed before the test object could again be seen was noted. The test with each object was repeated at least three times with rest intervals of one minute between each test. The results obtained with test objects of different sizes were charted, the ordinate indicating the size of the test object and the abscissa the duration of the refractory period. The curves of two individuals are shown in the following figures:

In figure 1 the curve in subject X and in figure 2 the curve in subject Y are shown. Both figures show that for any size of test object the duration of the refractory period was always shorter in binocular vision after binocular stimulation, *B*, than in monocular vision after monocular stimulation, *MD* and *Md*. In general, the duration of the refractory period became progressively shorter as the size of the test object was increased, and the curve became more or less vertical if the test object was

[1] This work was carried on under a grant from the John and Mary R. Markle Foundation.

15 mm. or larger, i.e., with a test object of 15 mm. or more, the duration of the refractory period remained fairly constant.

The curves of two individuals, X and Y, are compared in figures 3, 4 and 5.

The results of monocular stimulation upon the vision of the *dominant* eye are shown in figure 3. The acuity of vision of the two individuals was not the same: the threshold of X was an object of 8 mm. and that of Y was an object of 5 mm. For any size of test object the duration of the refractory period for X was always longer than that for Y because of this

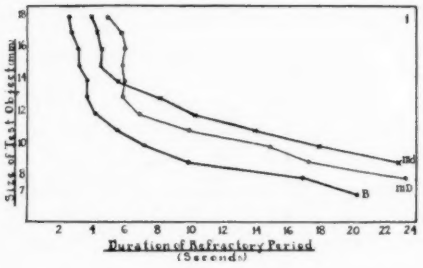

Fig. 1

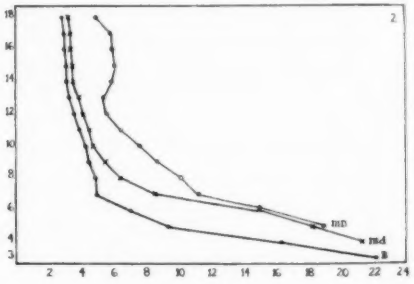

Fig. 2

Fig. 1. The effect on visual fatigue of increase in the size of the test object in subject X.

B = Binocular fatigue after binocular stimulation.

MD and *Md* = Monocular fatigue after monocular stimulation of the dominant and non-dominant eye.

The ordinates show the size of the test object in millimeters; the abscissas show the duration of the refractory period in seconds.

Fig. 2. The effect on visual fatigue of increase in the size of the test object in subject Y.

B = Binocular fatigue after binocular stimulation.

MD and *Md* = Monocular fatigue after monocular stimulation of the dominant and non-dominant eye.

The ordinates show the size of the test object in millimeters: the abscissas show the duration of the refractory period in seconds.

difference in visual acuity. With increasing size of the test object, the curves run fairly parallel until an object of 11 mm. for X and an object of 7 mm. for Y are reached. Thereafter the curves approach each other. When an object of 13 mm. was used for both X and Y, the curves became vertical and the duration of the refractory period for both individuals was almost the same and remained fairly constant with successive test objects 13, 14, 15, 16, 17 and 18 mm. in size.

The curves for the *non-dominant* eye are shown in figure 4. The threshold acuity object for X was 9 mm. and for Y was 4 mm. The duration of

the refractory period for X was always longer than that for subject Y. The curves run fairly parallel until for X, an object of 14 mm., and for Y, an object of 8 mm. is reached. From here on the curves approach each other. But as in figure 3, this is due more to the continued progressively shorter time for X than for Y. This indicates that the increase in the size of the test object was of significance for the vision of X (who had lower visual acuity) for a longer time than for Y. For subject X the curve was vertical with objects of 15 mm. to 18 mm., and for Y, with objects of 14 mm. to 18 mm. The other characters of the curves for the non-dominant eye are similar to those for the dominant eye, but the vertical parts of the curves are more nearly the same for X and Y for the dominant than for the non-dominant eye.

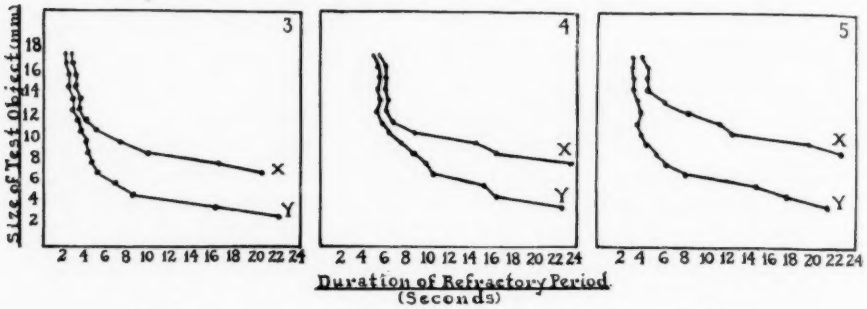

Fig. 3 Fig. 4 Fig. 5

Fig. 3. The curves of two subjects, X and Y, compared showing the effect of monocular stimulation upon monocular fatigue of the dominant eye. For details see text.

Fig. 4. The curves of two subjects, X and Y, compared, showing the effect of monocular stimulation upon monocular fatigue of the non-dominant eye.

Fig. 5. The curves of two subjects, X and Y, compared, showing the effect of binocular stimulation upon binocular fatigue.

In figure 5, the curves for the two eyes (binocular vision after binocular stimulation) are shown. In general, the characters of the curves are like those obtained for each eye separately, but the similarity is greater between the monocular curve of the dominant eye and the binocular curve.

The curves are fairly parallel until an object of 10 mm. for X and of 7 mm. for Y are reached. The curves then approach each other. With increase in size of the test object from 13 mm. to 18 mm. the curves are vertical, i.e., the duration of the refractory period remains fairly constant. For an object of 8 mm., the duration of the refractory period for X was 16.8 seconds and for Y 4.8 seconds; for an object of 10 mm., 7.4 seconds for X and 4.2 seconds for Y; for an object of 12 mm., 4.3 seconds for X and 3.7 seconds for Y. This shows that for a relatively small test object the differ-

ence in the duration of the refractory periods for the two individuals was 12 seconds; for a larger object it was 3.2 seconds, and for a still larger one it was only 0.2 second.

1. *The effect of monocular stimulation.*[2] The length of the period during which the test object was invisible by the same eye varies directly with the intensity of the stimulating light and the length of time of the exposure, and inversely with the intensity of illumination of the test object. These results represent the effects of monocular stimulation upon monocular vision, which we have called the effect of 1 on 1.

2. *The effect of binocular stimulation.* The length of the period during which the test object is invisible when viewed by the two eyes varies directly with the intensity of the stimulating lights and the length of time of the exposure to them, and inversely with the intensity of illumination of the test object. The results represent the effects of binocular stimulation upon binocular vision (the effect of 2 on 2).

3. If both retinae are simultaneously stimulated by light (binocular stimulation) but the test object is then viewed with only one eye (monocular vision), the relations between intensity and duration of stimulation and of the intensity of illumination of the test object, are similar to those found in 1 and 2. The results represent the effects of binocular stimulation upon monocular vision (the effect of 2 on 1)

4. The duration of the refractory phase (i.e., the period during which the test object is invisible) is always longer when the retina of one eye is exposed to the stimulating light and the test object is viewed with that eye alone, than when both eyes are simultaneously exposed to lights of the same intensity and the test object is viewed with both eyes. In other words, the duration of the refractory phase is always *longer* for monocular vision after monocular and ipsilateral stimulation than for binocular vision after binocular stimulation, i.e., 1 on 1 is longer than 2 on 2.

5. If both eyes are exposed to the stimulating lights but the test object is then viewed with only one eye, the duration of the period during which the test object is invisible is always *shorter* than in monocular vision after monocular and ipsilateral stimulation and *longer* than in binocular vision after binocular stimulation,—i.e., the duration of 2 on 1 is shorter than 1 on 1 and longer than 2 on 2.

In summary with a fixed intensity of illumination of the test objects and a fixed intensity and duration of exposure of the retina to a brighter light, *the duration of the refractory phase is longest in monocular vision after monocular and ipsilateral stimulation, shorter in monocular vision after binocular stimulation, and shortest in binocular vision after binocular stimulation.* These relations are not dependent upon pupillary contraction,

[2] For the sake of brevity, the term "stimulation" is used to indicate that the retina was exposed to light.

and were found when an artificial pupil, of 2 to 4 millimeters, was used or if the pupils had been dilated by a mydriatic. Under these different conditions the absolute figures for the duration of the refractory phase varied but the relative results were the same.

In all probability, the explanation for these results is the following:

1. During the exposure of one eye to a bright light, the retinal cells used for monocular vision by the same eye are affected for X seconds. When the test object is viewed during this refractory period, whatever impulse is sent to the brain is insufficient to reach the cortex and consciousness.

2. During the exposure of each of the two eyes to the same bright light as in 1, double the number of retinal cells are affected as in the first instance.

During stimulation of the two eyes by formed light, the two images are fused and appear as a slightly larger image a little further away from the eyes, and the brightness is greater than that of the image to each eye alone. The brightness of the fused image is, however, less than double that of the image to each eye alone. For with the same amount of light, the brightness of an object is somewhat less for a larger than for a smaller image. The same is true for the fusion of the images on the two retinae when a single object is viewed by the two eyes: the object appears slightly larger and definitely brighter than when it is viewed with one eye.

In either case, fusion of the two images is not retinal but is central and any changes in function that occur as the result of fusion must also be central.

These facts indicate that less impulse *from each retina* is required by the brain in binocular than in monocular vision. This is true both for distance vision of a single object by the two eyes with fusion of the retinal images and for near vision of two objects similar in size and brightness, one viewed by each eye, with fusion of the two images. For these reasons in binocular vision after exposure of *both eyes* to a brighter light than that which illuminates the test object, the duration of the refractory period during which the object is invisible, must be shorter than in *monocular* vision after *monocular* stimulation with light of the same brightness.

In *monocular* vision the cerebral visual centers require more impulse from the retina than is required from each eye in binocular vision. This explains the fact that the effect of binocular stimulation upon monocular vision is more prolonged than the effect of binocular stimulation upon binocular vision and shorter than the effect of monocular stimulation upon monocular vision. In what follows, these relations are illustrated by the use of theoretical values:

a. If we call the demand upon the retina required for monocular vision of a threshold test object as having a value of 5 units of retinal function

requiring 1 second of time, and the demand made upon each retina in binocular vision as 3 units requiring 0.6 second; and *b*, if we give the change produced by exposure of one eye to a bright light a value of 15 units of function, and that resulting from the exposure of each of the two eyes to the light a value of 9 units; and furthermore, if it be predicated that *c*, recovery of function, after both monocular and binocular stimulation by the bright light occurs at a constant rate, say 4 units per second,[3] then:

1. In monocular vision after monocular stimulation, 3.75 seconds will elapse before the retinal equilibrium has been restored and after this 5 units of retinal function or 1 second are required before the test object can be seen, i.e.,

$$\tfrac{15}{4} + 1 = 3.75 + 1 = 4.75$$

2. For monocular vision after binocular stimulation the formula will be

$$\tfrac{9}{4} + 1 = 2.5 + 1 = 3.5$$

3. For binocular vision after binocular stimulation, the formula for each eye will be

$$\tfrac{9}{4} + 0.6 = 2.5 + 0.6 = 3.1$$

Therefore, in the instance given above, the duration of the refractory period is 4.75 seconds for monocular vision after monocular stimulation; 3.5 seconds for monocular vision after binocular stimulation and 3.1 seconds for binocular vision after binocular stimulation.

[3] That the *relative* values of *a* and *b* are correct for a given size of test object was shown by actual experiments. The correctness of the assumption *c* was demonstrated by tests in which the smallest test object that could be seen by the two eyes after binocular stimulation by a brighter light was compared to the size of the smallest test object that could be seen by one eye after monocular stimulation. In the latter instance, the test object was always larger, and a definite period of time elapsed before the test object at once visible in binocular vision after binocular stimulation could be seen by one eye alone.

THE EFFECT OF DIET ON THE SURVIVAL OF ADRENALECTOMIZED RATS

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The physiology of the adrenal glands has been recently found to be intimately connected with salt metabolism. Cortical insufficiency is greatly ameliorated by sodium salts (cf. Gaunt, Tobin and Gaunt, 1935), while potassium salts exert a very noxious effect (Allers, Nilson and Kendall, 1936; Zwemer and Truszkowski, 1936). However, these have been, with exceptions to be discussed below, the only dietary factors to be explored in detail. Scott (1923) and Agate and Zwemer (1935) indicate that adequate diets are important variables in determining the total mortality of rats after adrenalectomy, but neither indicates precisely how. Accordingly an investigation was projected on the effects of various diets on the survival of adrenalectomized rats.[1]

Cleghorn, Cleghorn, Forster, and McVicar (1936) obtained results, independently confirmed in this study, that indicate clearly that bread leads to a survival of adrenalectomized rats far longer than that secured with other diets. Typical of their results are the following: if rats were fed on meal cake only, 100 per cent of a group of 19 rats died within ten days; if bread was added to this diet, 43 per cent of a group of 17 rats died within 31 days.

Furthermore, it seemed possible that the reliability of the rat in studies of cortico-adrenal insufficiency might be dependent on the diets fed the animals. Variables known to affect the mortality of adrenalectomized rats are numerous. Colony differences in susceptibility, presumably due to accessory tissue, have been twice demonstrated by Gaunt (1933, 1935). Freed, Brownfield and Evans (1931) have suggested that careful removal of the pedicle along with the gland leads to high mortality. The conditions under which the animals are kept, particularly that of temperature have often been stressed as important. Agate and Zwemer (1935) consider meticulous asepsis important. In spite of these obstacles to consistent work, a large number of workers have recently reported high mortalities in the rat (cf. Gaunt, 1933; Agate and Zwemer, 1935), and

[1] Dr. B. O. Barnes suggested this problem. It is a pleasure to acknowledge his aid during its course. A preliminary report has been made (Swann, 1935).

many have used this animal in cortico-adrenal studies. Most agree on mortalities of about 90 per cent or greater. A vigorous dissent to such findings has recently been made by Agate and Zwemer (1935). They ascribe their low mortalities—only about 10 per cent—to the excellent living conditions and health of their rats and to the carefulness of the operation. They tested four colonies, all yielding about the same mortality.

METHODS. All the variables discussed above which are known to affect the mortality of adrenalectomized rats were kept as nearly as possible constant, the only varying factor being the diets fed the rats. Rats from the laboratory colony were used, with one exception which will be mentioned below. The operation was performed by the author, complete bilateral removal of the glands within their capsules along with the pedicles being made by the dorsal route. Ether anesthesia was employed. All rats, again with exceptions which will be discussed below, were 28 days of age.

The diets to be tested were fed to groups of 21 or more rats from weaning at 21 days of age until death ensued. At 28 days of age the operation was performed. After the operation, the animals were removed to a constant temperature room kept at 30°C. Records were kept of the sex, condition, operation, and period of survival of each animal. In some, daily weight and temperature changes were followed. Each litter of rats was so marked as to be identifiable. The rats were kept on clean sawdust in large cages; tap water was always available to the animals in large bowls.

RESULTS. The import of the data is best comprehended by means of graphs. In each of these, the cumulative percentage of deaths within a single dietary group is plotted against the number of days after adrenalectomy. From these, it can be appreciated when the animals were dying rapidly and what the incidence of death was within a single group. When such a curve is flat, it indicates that no deaths occurred; when steep, that a large portion of the group was dying. The percentage of a group dying on any given day can readily be computed by finding the difference between the percentage dead at the beginning and at the end of the day.

The following diets were tested:

No. 1

67% cracked corn
33% powdered skim milk } ad lib.
21% fresh whole milk
59% bread
4% raw beef lung
16% water } About 10 grams per rat per day
Lettuce

No. 2

60% fresh whole milk
40% bread
1.46 mgm. $FeCl_3$
0.013 mgm. $CuSO_4$ } Per rat per day
Lettuce

No. 3

Bread, dried and ground fine
Lettuce

No. 4

77% starch
14% casein
5.8% dried yeast
3.2% Osborne and Mendel salt mixture
Lettuce

No. 5

75% starch
14% casein
5.8% dried yeast
5.2% O. and M. salt mixture
Lettuce

No. 6

"Fox-chow"—Ralston Purina Co.
Lettuce

No. 7

50% butter
14% lard
25% casein
5% cod liver oil
6% O. and M. salt mixture
Lettuce

No. 8

91% casein
5.8% dried yeast
3.2% O. and M. salt mixture
Lettuce

No. 9. Sherman "optimal" (1932)

67% ground whole wheat flour
33% powdered whole milk
Lettuce

No. 10. Scott diet (1932)

60% hominy
15% rolled oats
14% dried meat scrap
10% powdered whole milk
1% salt
Lettuce

No. 11. Agate and Zwemer diet (1935)

37.5% yellow corn meal	ad lib.
37.5% ground whole wheat	ad lib.
16% linseed oil meal	ad lib.
5% casein	ad lib.
2% ground alfalfa	ad lib.
1.5% bone ash	ad lib.
0.5% NaCl	ad lib.
67.5% ground whole wheat	Fed as a paste
15% casein	Fed as a paste
10% whole milk powder	Fed as a paste
5% butter	Fed as a paste
1.5% $CaCO_3$	Fed as a paste
1% NaCl	Fed as a paste

Lettuce and meat scraps

No. 12

"Pel-ets"—Nowak Milling Co.
Lettuce

Diets 4, 5, 7 and 8 are each of a special type. No. 4 (Osborne and Mendel, 1920; Levine and Smith, 1927) is high in carbohydrate but low in fat. No. 7 (Osborne and Mendel, 1921a; Levine and Smith, 1927) is high in fat, but low in carbohydrate. No. 8 (Osborne and Mendel, 1921b) is high in protein but low in fat and carbohydrate. No. 5 is high in salts.

Diets 10 and 11 have been used by workers reporting very low mortalities in the rat after adrenalectomy. Diets 6 and 12 are commercial diets. No. 12—"Pel-ets"—is meant for rabbits, not rats, but it has been successfully used with mature rats. Number 6 has been found satisfactory for rats of any age.

An effort was made, no. 3 excepted, to have all these diets adequate. Except for no. 3, all have been reported adequate for mature rats. Our 28-day rats however were, in the case of some diets, not in a state of

good nutrition when they came to operation. The offending diets on this point were nos. 3, 7, 8 and 12; they cannot, therefore, be considered adequate diets. Also no. 4 has been found inadequate for growth over a long period; for a month, however, even these young rats do well on it. All the rest of the diets were thought to be satisfactory.

In figures 1 and 2 the mortality rates of each group of animals on these diets are presented, along with the number of animals tested. Each of these rates falls into one of two types: the one has an early and rapid death rate, 70 per cent or more of the deaths in all groups having occurred by the ninth day after adrenalectomy and 95 per cent or more by the twenty-first day. Diets 4 through 12 yield this type of mortality curve.

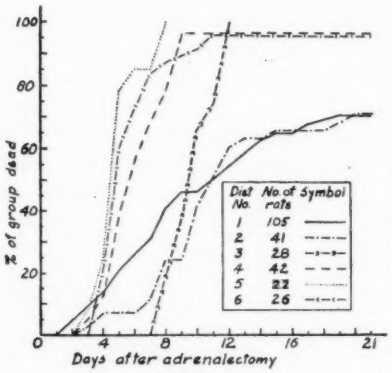

Fig. 1

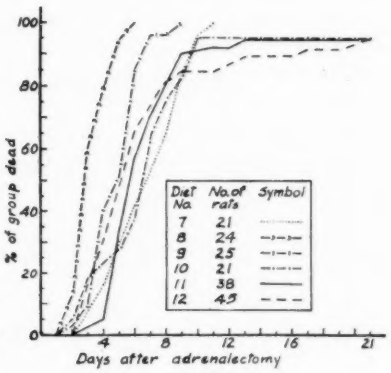

Fig. 2

This type of curve will subsequently be referred to as a "short-survival" mortality curve.

The second type of curve—to be called the "long-survival" type—has a less rapid rate: by the ninth day after adrenalectomy about 30 per cent have died and by the twenty-first day an average of 75 per cent. Diets 1, 2 and 3 are of this type. The median death of the former group occurs on the fifth day, that of the latter group on about the ninth day—an average survival by the latter group almost twice that of the former.

Although there is considerable variation in each of these two general types of curve, the data are not considered adequate for any attempt to find a significant difference between any of the diets in either of the two main groups. More animals would be needed to settle such a difference, if it exists.

It seemed desirable to ascertain whether other colonies would react similarly to this dietary difference in susceptibility to adrenalectomy. Four colonies were tested. The number of rats used and the results are

presented in figure 3. Stock I consisted of albinos from the laboratory colony. Stock II consisted of mixed rats from the Department of Psychology, University of Chicago. Stock III and stock IV, albinos and McCollum type rats respectively, were obtained from different dealers. No colony difference was found, each colony yielding mortality curves similar to the two types obtained with the main test colony.

The two types of diet were tested on mature rats, no. 1 as representative of long-survival diets and no. 4 as representative of short-survival diets. In figure 4 the results are shown. The difference in the mortality rate of mature rats is marked, 90 per cent of those on the short-survival type of diet having died by the twenty-first day whereas 13 per cent of those on the long-survival type died in the same period. It appears from this set of data that younger animals are considerably more sus-

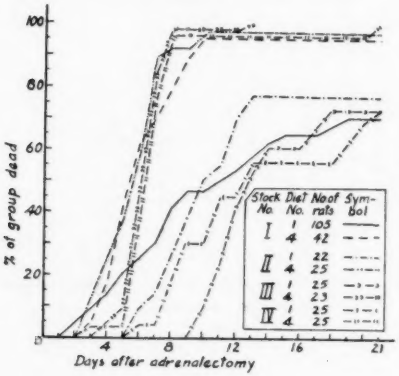

Fig. 3

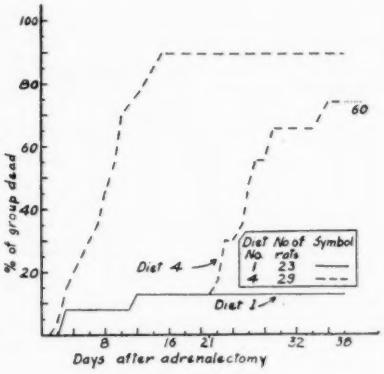

Fig. 4

ceptible to adrenalectomy on either type of diet. Mature rats on the long-survival type of diet survive the operation as a group quite well, maintaining their weight and, apparently, good health.

In order further to prove that it is a dietary effect which leads to this difference in mortality rates, the survivors of groups on diets 1 or 2 were placed at 21 days after adrenalectomy on a short-survival type of diet, i.e., on no. 4. Controls were also made to determine how the mortality rate of rats kept on the survival type of diet changed subsequent to 21 days after operation. Figure 4 shows that while no mature rats died during the fourth week after adrenalectomy if kept on the long-survival type of diet, most of them died in about this same period if changed to the short-survival type of diet. The same holds true for young rats. A group of them so treated showed a final mortality of 97 per cent.

Discussion. That the long-survival type of diet is by no means a

complete replacement therapy is indicated by a variety of facts: in the first place, 75 per cent of young animals and 18 per cent of old die under this treatment, indicating that it is not adequate replacement for all animals. Secondly, the growth of 28-day adrenalectomized rats on long-survival diets is much retarded. At 60 days of age, 32 days after adrenalectomy, they averaged 111 grams, about 30 per cent underweight.

The common factors in diets 1 and 2, both long-survival types of diet, are bread and milk. When bread alone is fed (diet 3), the effect of the operation is delayed. The rats do not die until 7 days after operation, but then deaths in the group occur rapidly until on the twelfth day, 100 per cent have died (fig. 2). This is somewhat different from the long-survival type of curve; possibly the relative adequacy of the diets will account for the difference.

We are still uncertain why it is that bread exerts this beneficial effect after adrenalectomy. Cleghorn et al. (1936) consider that the effect cannot be ascribed to sodium chloride alone. With this we are in agreement, but the case is not yet clear. When the sodium and potassium intake of rats on the two types of diet are measured, the following approximate figures are found:

A rat on diet 3 (long-survival) consumes daily 74 mgm. Na and 20 mgm. K
A rat on diet 6 (short-survival) consumes daily 15 mgm. Na and 44 mgm. K

Not only is the sodium intake of diet 3 comparatively high, but also the potassium intake is comparatively low. The reverse is true in the case of both cations in a short-survival type of diet. Because in adrenal insufficiency sodium exerts a beneficent effect, but potassium a noxious effect, the effect of these diets may be due to their sodium and potassium contents. Indeed, when the sodium intake of rats on diet 6 (short-survival type) was raised to 74 milligrams by adding sodium chloride to the drinking water, a group of 24 rats died at the long-survival rate.

Both of these facts might seem to indicate that the beneficent effect of bread is ascribable to its relative sodium and potassium content. However, we have found that salt will not produce such effects in the short-survival types of diet unless the salt is in the drinking water, i.e., salt did not prolong the life of a group of 31 rats when added dry to dry diet no. 6. Apparently it is a matter of fluid balance as well as salt and other balances.

Two of the diets here tested have been used by other workers, each reporting low mortalities after adrenalectomy. These diets are nos. 10 and 11, those of Scott (1923) and Agate and Zwemer (1935); both of them yielded mortalities of 95 per cent in our hands. Whatever may be the cause of the discrepancy in results, it does not appear to be the diets employed.

When the odd 25 per cent of rats surviving all diets (cf. fig. 4) were brought to autopsy, there were almost invariably found at the head of the kidney large pieces of adrenal tissue, often of the size of a normal gland. Consequently we have been able to ascribe such long survivals to these pieces, and not to an abnormal resistance of the rat to adrenal ablation. Such pieces are presumably due to hypertrophied tissue, resulting either from accessory tissue or incomplete removal of the gland.

It has also been found that the effect of bread-containing diets on adrenalectomized rats cannot be duplicated on dogs. Three dogs, well habituated to a diet of bread and milk, were adrenalectomized in a two-stage operation. They died, respectively, in 2, 2 and 4 days, with controls on meat diets dying in about the same period after the second operation was performed.

SUMMARY

The susceptibility of groups of about 25 28-day rats to adrenalectomy has been found to be quite constant on a number of diets, including the Sherman "Optimal," "Fox-chow," the Agate and Zwemer combination (1935) and others. Mortalities of 95 per cent or greater were obtained, with the median death occurring on about the fifth day after operation. However, one substance, bread, leads to both a prolongation of life and a smaller total mortality. About 72 per cent of rats on bread-containing diet succumb to the operation, with the median death occurring on the ninth day.

Mature rats show a clearer difference in this dietary effect. Whereas 13 per cent die on a bread-containing diet, 90 per cent die on a bread-less diet.

Four colonies were tested, all yielding similar results. In all, 704 rats have been employed.

When those rats which had survived on bread-containing diets for 21 days were changed to bread-less diets, they died within a week. This is taken as a further indication that the differences in mortality rates are due to dietary variables.

Bread will not, however, lengthen the life of adrenalectomized dogs.

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CONTENTS

Tissue Extracts and Blood Coagulation. *Forrest Ramon Davison* 633
Effects of Faradic Stimulation of the Cerebral Cortex on Limb and Renal Volumes in the Cat and Monkey. *Harold D. Green and Ebbe C. Hoff* 641
A Study of the Blood Sugar Raising Substance in the Urine of Diabetic and Non-Diabetic Patients. *S. Carl Werch and Samuel S. Altshuler* 659
Positive Friedman Tests in the Pregnant Rhesus Monkey, Macaca Mulatta. *G. W. D. Hamlett* 664
The Direct Determination of the Renal Blood Flow and Renal Oxygen Consumption of the Unanesthetized Dog. *Morton F. Mason, Alfred Blalock and Tinsley R. Harrison* ... 667
Effects of Cortico-Adrenal Extract on Growth and Sexual Activities. *Oscar Garth Fitzhugh* 677
A Study of the White Blood Cell Picture in Six Young Men. *Beverley R. Kennon, III, Mary E. Shipp and Duncan C. Hetherington* 690
Blood and Muscle Lactic Acid in the Steady State. *Jacob Sacks and Wilma C. Sacks* 697
The Effect of Variations in Total Calcium Concentration upon the Coagulation Time of Blood. *Marian M. Crane and Heyworth N. Sanford* 703
Effect of Motion on the Electrical Conductivity of the Blood. *E. Sigman, A. Kolin, L. N. Katz and K. Jochim* 708
Extirpation of the Neocerebellar Cortex without Eliciting So-called Cerebellar Signs. *Allen D. Keller, Raymond S. Roy and Wilton P. Chase* 720
The Effect of Hemorrhage, Intestinal Trauma and Histamine on the Partition of the Blood Stream. *Alfred Blalock and Sanford E. Levy* 734
The Simultaneous Measurement of Renal Blood Flow and the Excretion of Hippuran and Phenol Red by the Kidney. *Kendall A. Elsom, Phyllis A. Bott and Arthur M. Walker.* 739
The Effect of Remote Leads upon the Form of the Recorded Electrocardiogram. *A. S. Gilson and G. H. Bishop* 743
A Study of the Relation of the Spleen to Erythropoiesis and Red Cell Destruction in the Guinea Pig. *Albert S. Gordon and William Kleinberg* 757
Acid Inhibition of the Intestinal and Intragastric Chemical Phases of Gastric Secretion. *Charles M. Wilhelmj, H. H. McCarthy and Frederick C. Hill* 766
The Influence of Oil Enemas on Colon Motility in the Dog. *E. L. Borkon and R. D. Templeton.* 775
The Control of the Large Intestine by the Decentralized Inferior Mesenteric Ganglion. *Hampden Lawson and J. P. Holt* 780
The Effect of a Lysine Deficient Diet on the Estrous Cycle. *Paul B. Pearson* 786
Some Neural Components of the Visual Response. *Charles A. Elsberg and H. Spotnitz* 792
The Effect of Diet on the Survival of Adrenalectomized Rats. *H. G. Swann* 798
Index 807

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PHYSIOLOGICAL REVIEWS

Contents of Volume 16, 1936

J. BARCROFT: Fetal Circulation and Respiration
W. J. V. OSTERHOUT: Electrical Studies on Large Plant Cells
W. O. FENN: Electrolytes in Muscle
A. N. DRURY: Physiological Activity of Nucleic Acid Derivatives
R. HÖBER: Membrane Permeability to Solutes in Its Relations to Physiology
L. B. AREY: Mechanism of Wound Healing
H. DAVIS AND A. FORBES: Chronaxie
A. P. KRUEGER: The Nature of Bacteriophage and Its Mode of Action
C. G. KING: Ascorbutic Acid and Vitamin C
H. J. DEUEL: Metabolism of Fructose and Galactose
W. O. NELSON: Lactation
D. B. DILL: Economy of Muscular Activity
R. S. ALCOCK: The Synthesis of Proteins in Vivo
JOHN H. FERGUSON. The Blood Calcium and the Calcium Factor in Blood Coagulation
H. G. WOLFF: The Cerebral Circulation
E. W. H. CRUICKSHANK: Metabolism of Cardiac Muscle
H. R. ING: Curariform Action of Onium Salts
L. EARLE ARNOW: Effects Produced by the Irradiation of Proteins and Amino Acids.
G. BOURNE: The Rôle of Vitamin C in the Organism as Suggested by Its Cytology
E. PONDER: The Kinetics of Hemolysis

PHYSIOLOGICAL REVIEWS

Tentative Contents of Volume 17, 1937

S. R. M. Reynolds: Control of Uterine Motility

E. E. Nelson and H. O. Calvery: The Present Status of the Ergot Problem

Raphael Isaacs: Formation and Destruction of Red Blood Cells

Leslie Hellerman: The Reversible Inactivation of Hydrolytic Enzymes

C. B. Huggins: Physiology of Bone

Selig Hecht: Chemistry of Vision and Photoreception

H. K. Hartline: Electrical Studies of Visual Mechanisms

Sarah S. Tower: Degeneration in Skeletal Muscle

F. R. Winton: Physical Factors Involved in the Activites of the Mammalian Kidney

Leo Loeb: Physiological Old Age

V. E. Henderson and M. H. Roepke: Drugs Affecting Parasympathetic Nerves

W. G. MacCallum: Physiological Pathology of the Prostate

A. E. Severinghaus: Cellular Changes in the Anterior Hypophysis With Special Reference to its Secretory Activities

M. C. Bourne: Metabolic Factors in the Production of Cataract

J. R. Murlin: Some New Factors of Energy Metabolism

G. W. Bartelmez: Menstruation

D. W. Bronk: Unitary Analysis of Reflex Activity

P. Bard: Functions of the Hypothalamus

H. S. Liddell: Recent Contributions to the Physiology of the Conditioned Reflex

W. H. Chambers: Undernutrition and Carbohydrate Metabolism

J. H. Northrop: The Formation of Enzymes

William Bloom: Cell Differentiation in Tissue Cultures

G. L. Brown: Transmission at Nerve Endings by Acetylcholine

A. Rosenblueth: The Transmission of Sympathetic Nerve Impulses

J. C. Eccles: Synaptic and Neuromuscular Transmission

A. G. Bills: Fatigue in Mental Work

H. H. Woollard: Specificity in Structure and Function of Nerve Endings of the Skin

Helen Tredway Graham: The Significance of the Potentials Manifested During Nervous Activity

Hugo Krueger: The Action of Morphine on the Diegestive Tract

W. C. Rose: Nutritive Significance of Amino Acids

Phyllis Tookey Kerridge: Physiology of Hearing and Speech

A. Baird Hastings: Electrolyte Equilibria in the Blood and Body Fluids

F. Gowland Hopkins: Glutathione

William E. Anderson and H. H. Williams: Rôle of Fat in the Diet

C. P. Richter: The Psycho-Galvanic Reflex

E. S. G. Barron: Cellular Respiration

R. K. Burns: Experimental Transformations of Sex in Vertebrates

J. J. Morton and W. J. M. Scott: Sympathetic Vasoconstriction in Normal and Pathological Arteries of the Extremities

Carl L. Voegtlin: Biochemistry of Malignant Tissues

F. C. Koch: The Male Sex Hormones